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THE LABORATORY EVALUATION OF A METHOD FOR ENHANCING THE KINETICS OF ACTIVATED SLUDGE TREATMENT PLANTS

S. J. Sedita

Houston Research Institute, Incorporated

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Cell disruption was followed by TOC increase and cell number decrease, and on the average resulted in a significant reduction in cell numbers both at 20 minutes and at one hour.

The use of disrupted cells may have some potential in waste treatment as demonstrated by substrate removal with sonically disrupted sludge or the supernate from sonically disrupted sludge.

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Activated sludge from the Bellaire, Texas plant was acclimated to five different test compounds, 2,4-Dichlorophenol, Linear Alkylate Sulfonates, Nitrilo-triacetic Acid, Ethylene dinitrilotetraacetic Acid and Ethylene glycol. The test compounds were then incorporated into waste water influent to the Bellaire plant and fed to the acclimated sludge which had been mechanically disrupted by sonic energy, to varying degrees.

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SECTION 1

CONCLUSIONS

1. Sonication releases significant amounts of soluble materials in the form of Total Organic Carbon from concentrated sludge.
2. Sonication kills a significant portion of the micro-organisms found in concentrated sludge.
3. The substrate removal activity of disrupted sonicated sludge is not significantly different from undisrupted sludge having a thousand times the cell concentration.
4. The cell free supernate from disrupted sludge may have substrate removal activity up to 10 times that of normal sludge, on a solids content basis.
5. Further exploration of the relationship between substrate removal and solids concentration may result in design data for enhancing waste treatment systems.
6. Cell free supernate may effectively be utilized by addition to waste water influent, prior to mixing with return sludge.
7. It may be possible to obtain a significant increase in throughput capacity by using a combination of normal return sludge and disrupted sludge or supernate.

SECTION II

RECOMMENDATIONS

It is recommended that a pilot plant be constructed and further studies made to demonstrate the potential for application of disrupted sludge to improve efficiency of existing waste treatment facilities.

Such studies must include:

1. The effect of solids concentration on the removal rate.
2. The removal of disrupted particulate matter prior to mixing sonicated sludge with undisrupted sludge.
3. The efficiency of other mechanical methods of disrupting sludge.

An approach that should be investigated is the addition of only the supernate to the influent waste water prior to its mixing with the return sludge. In this instance, provision would be made to separate and hold sufficient supernate prior to addition. The fate of the disrupted particulate material should also be investigated.

An alternate approach is the addition of the disrupted particulate material to the return sludge to take advantage of the membrane bound enzymes and to add the supernate to the incoming waste water to take advantage of solubilized enzymes.

SECTION III

INTRODUCTION

Scope

A laboratory study of the activated sludge process was carried out. This project was financed under a contract from the Environmental Protection Agency (EPA), Project Code 17050 GIU.

The basic purpose of this program was to determine the technical feasibility that the disruption of a portion of the microbial cells in the return sludge will enhance the operating efficiency and capacity of municipal activated sludge plants.

The specific objectives were to:

1. Evaluate on a laboratory scale the effect of return sludge disruption on the rate of biological oxidation of specific sewage components.
2. Examine the effect of cell disruption on the physical and chemical characteristics of activated sludge operation, by determining the net effect of sludge disruption on the Total Organic Carbon (TOC) concentration of settled, bench treated waste.
3. Evaluate the effects of varying degrees of cell disruption upon the oxidation rate.
4. Evaluate the effects of varying concentrations of substrates on the oxidation rate.
5. Evaluate the effect of disruption on the activated sludge itself with respect to cell numbers, and Total Organic Carbon (TOC) as a measure of disruption.

6. To provide data for design and construction of an on-stream pilot plant.

Background

The usual treatment processes for either domestic or industrial wastes employ enzymes. The production of these enzymes is accomplished in the waste itself through the growth of micro-organisms. Enzyme production in this situation is proportional to the rate of microbial growth. Depending on the enzyme system involved, its activity may be intracellular or extracellular. Enzymes confined to the interior of the microbial cell cannot act on insoluble compounds, and act only poorly on slightly soluble compounds.

The concept of activated sludge implies that such a sludge is already conditioned or acclimated to the various wastes which it is used to treat. Consequently, one of the rate limiting steps is contact between the waste and the appropriate enzyme(s). The rate of oxidation should increase if these enzymes are made immediately available for contact with the waste by removing the cell wall-cell membrane barrier.

This study was carried out to study the effects of mechanically disrupting the cells of an acclimated sludge, on the rate of oxidation of the system.

Disruption of Cells - Ultrasound (Damm, 1966. References 1 and 2) The effect of ultrasound on cells and microorganisms (and by extrapolation on activated sludge) has been studied largely in the presence of cavitation. Although cavitation is not the only mechanism by which the effects of ultrasound are manifested, we will restrict this discussion to effects occurring in the presence of cavitation.

The cavitation threshhold varies with the geometry of the sound field, the frequency, the chemical composition of the suspending fluid, temperature, viscosity and pressure. The relative rates at which various types of biological cells are destroyed is, however, constant over a wide frequency range for specified values of the other parameters. As long as 1% of the population is undamaged, then the rate of cell destruction is described by the expression:

$$\frac{dN}{dt} = -RN$$

where: N = cell concentration

t = time

R = rate constant.

The rate constant, R, can be considered a measure of cellular fragility. provided the physical conditions remain constant. Using the fragility of the human red cell as 1, it may be shown that larger cells tend to be more fragile, for example, Paramecium aurelia G is 16 times more fragile than human RBC's, whereas the common microorganism Escherichia coli is 0.15 times as fragile. Unfortunately, there is no simple relationship between cell size and fragility.

The primary problems encountered in cellular disruption may be listed as follows: (Damm, 1966, References 1 and 2)

1. Adequacy of disruption rate and power delivered to obtain maximal yield of soluble protein with minimum denaturation.
2. Control of local and total heating.
3. Mixing
4. Prevention of oxidation by atmospheric O_2 or NO_x.
5. Sample size
6. Effects of pH, ionic strength and specific buffer ions on the efficiency of cell disruption and on protein solubilization.
7. Determining the geometry and size of the container utilized for disruption.
8. Problems associated with aerosol prevention and sterility when using pathogenic or toxic microorganisms.

As stated previously, the efficiency of cell disruption will vary according to cell type. Bacteria are quite different in their breakage characteristics from yeast or mammalian cells. The initial event in disruption involves the abrasive cleavage of the cell wall or cell membrane by high-speed micro-bubbles of gas. The result is the release of the cellular contents containing both soluble and insoluble fragments. Further sonication breaks down the particulate material to give additional soluble protein. As a consequence, enzymes normally associated with particulate fractions of the cell are solubilized at a rate different than the actual rate of cell wall disruption.

Heat generation during sonication can be a serious problem, as without adequate provision for cooling there is likely to be sufficient total temperature rise to cause damage to sensitive enzymes.

Mixing problems occur during sonication only when fibrous materials or very heavy bacterial suspensions are being disrupted. If the cell suspension is kept below 10% wet weight of cells by volume, the mixing provided by the intense cavitation is usually sufficient.

Oxidation problems become serious mainly where the study of sulfhydryl enzymes is concerned. Free radical scavengers, such as cysteine, reduced glutathione, etc., may be used to alleviate this problem, or operation in an inert atmosphere may be considered, as the gas phase for efficient cavitation need not necessarily be O_2 or air.

Sample size may become a problem when it is desired to scale up laboratory studies to pilot or plant operation. On the laboratory scale from 5 ml - 300 ml, no real problem exists.

Considerable variations exist in the extraction of soluble protein as a function of pH, buffer or ionic strength, etc., in the use of glass beads as disruption aids, etc. Disruption aids (glass beads and diatomaceous earth) apparently function as a source of nucleation for bubble formation thus increasing the shearing action present in the solution. Such aids are useful in the disruption of resistant cells such as yeast or

mold fragments. With most bacteria, however, the net gain is not significant. Additives such as alumina or Celite^{R} frequently adsorb proteins and hence enzymes. pH and buffer effects on the efficiency of sonication are quite marked. Primarily, salts in solution promote the solubilization and stabilization of proteins. Additionally, there may be some osmotic effects on subcellular particles, e.g., microsomes, mitochondria, which are sensitive to osmotic changes. On the average, isotonic salt concentrations are advantageous. pH may vary within fairly wide limits, however, extremes should be avoided. A pH between 6.5 and 8.5 using a low ionic strength buffer is generally the most desirable. This parameter may be varied to suit the needs of the investigator. There is considerable evidence which suggests that the shape of the container exerts some influence on the rate of release of soluble protein and on the efficiency of cellular disruption. This is probably more a function of mixing than anything else since individual cells or other components are not directly exposed to the action of the probe tip for more than a second or two. In very large volumes, individual cells are not in the area of power output for a sufficient time period.

Aerosol formation and its prevention is difficult at best. For small volumes, 15-300 ml, a completely sealed and sterile container is possible, however, in dealing with large volumes this would seem an impossible task in a stationary, uncovered container. If sonication could be achieved in a pipe or holding tank, the problem would be solvable.

SECTION IV

EXPERIMENT FABRICATIONS AND MATERIALS AND METHODS

Reactor Design

Reactor design as specified in the Statement of Work was a two (2) liter continuous flow bench type reactor. The apparatus design finally chosen is shown in schematic form in Figure 1 and in the photograph in Figure 2.

Each compartment of the four compartment reactor represents a homogeneous, single stage stirred fermenter, (with aeration) of the type described by Herbert (1961).

Experimental Procedure

Each experiment was carried out simultaneously as follows:

Reactor A - undisrupted sludge

Reactor B - blended sludge (Waring Blender 4 min.)

Reactor C - blended sludge, sonicated to varying degrees

Reactor D - soluble fraction of blended sonicated sludge.

The reactors were operated at a flow rate of 5.5 ml/min. to give an average hydraulic retention time of approximately 6 hrs. (except in those cases where an extended retention time was investigated). Sampling was cumulative over 90 min. intervals, at which time the appropriate chemical analysis was carried out as well as recording of the volume, total suspended solids determination or other pertinent manipulation. The test compounds were studied by incorporating them into a 10 liter volume of wastewater from the Bellaire, Texas plant and using this mixture to feed the reactors at a constant rate and constant concentration of the test compound. The starting concentration

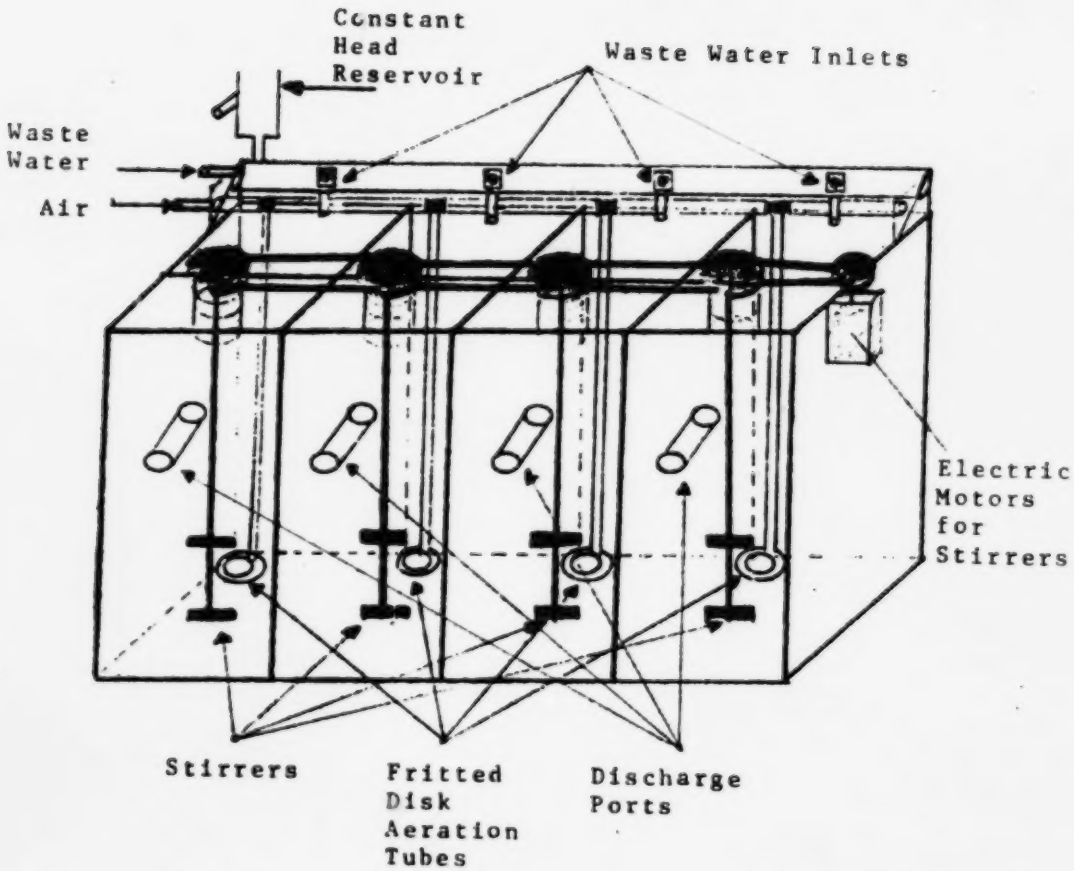

FIGURE 1. Schematic Drawing of 4 Place Reactor Used to Determine Effect of Disruption on Kinetics of Activated Sludge.

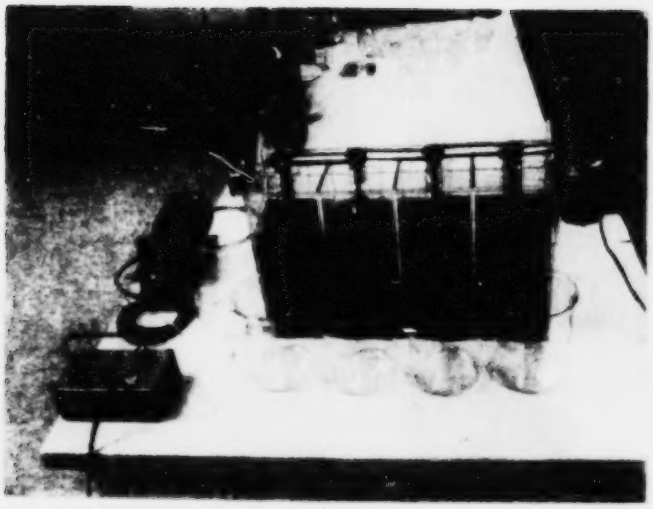

FIGURE 2. Photograph of 4 Place Reactor

of both the reactor and the feed water with respect to the test compound was always the same for a particular run.

Acclimation of Sludge to the Test Compounds

Return sludge from the Bellaire, Texas plant was utilized to develop a sludge acclimated to the various test compounds. Acclimation was accomplished by the draw and fill batch technique for each compound as follows:

1. 5 liters return sludge
2. 5 liters waste water influent plus test compound at highest concentration to be tested
3. Aeration at approximately 9 liters per minute
4. At 24 hour intervals the aeration was stopped. The mixed liquor suspended solids was allowed to settle for one hour. The supernatant liquid

was drawn off. An amount of waste water plus test compound equal to the volume of supernate was added.

5. Aeration was started again.

A variation of the above procedure was to start with a low concentration of the test compound and to increase, by discrete increments, the concentration of test compound until the desired concentrations were achieved. This was done with the more toxic compounds, e.g., 2,4-dichlorophenol.

Disruption of Microorganisms - Sonication Technique

Acclimated sludge was disrupted by sonication, using a Bronwill Biosonik III with a standard 3/4" diameter probe tip. The tip delivers 300 watts or 680 watts/in^2 or 105 watts/cm^2. The probe was operated at maximum output.

The geometry of the vessel was such that mixing due to cavitation was at a maximum, thus no external mixing was required (see Figure 3), temperature was controlled with a salt-ice bath so that the temperature of the sonicated material was never above 18C.

On the day of an experiment the total suspended solids of the appropriate acclimated sludge were determined. Then 1500 ml of the sludge were centrifuged (200 ml at a time) 10 minutes at approximately 3000 RPM in an International clinical centrifuge, Model CL. The sludge pellet was resuspended in 300 ml of the supernate to achieve a concentration factor of 5X.

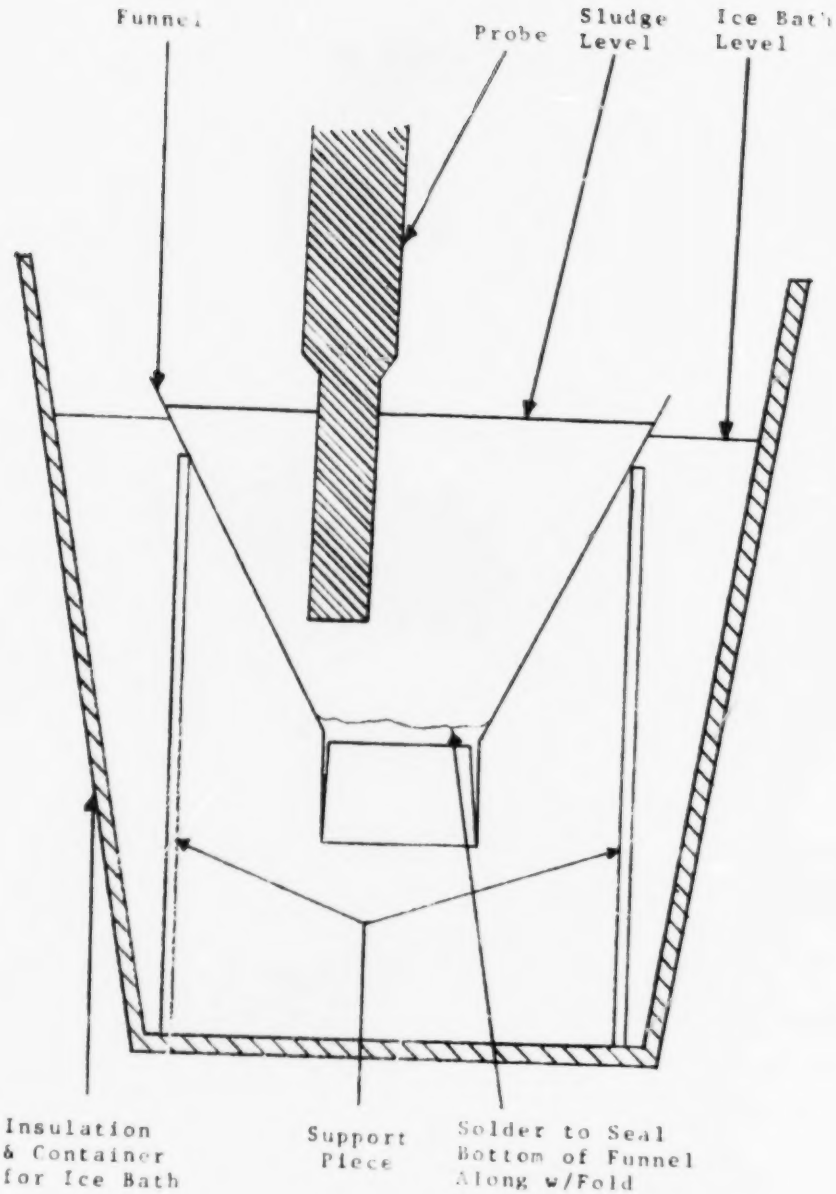

FIGURE 3. Schematic of Sonication Apparatus

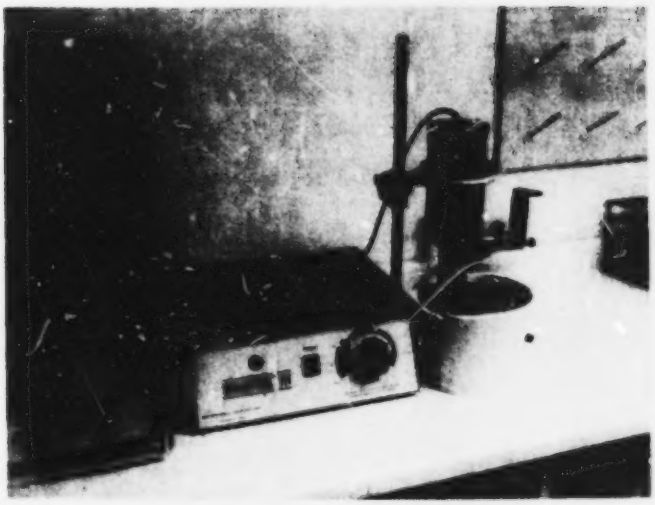

FIGURE 4. Photograph of Sonication Apparatus

The concentrated sludge was then placed into the sonication vessel in the salt-ice bath and allowed to chill for 15-20 minutes.

Sonication was carried out for 20 minutes (S-1) or 1 hour (S-2). Total Organic Carbon increase in the supernate as a function of sonication time was used as the measure of adequate sonication. Also, cell survival and dry weight determinations were used to follow cell disruption by sonication. Baker's yeast was also used to determine the efficiency of sonication. (See Figures 5 and 6.) After sonication, a volume of sonicated material equivalent to the amount required to achieve a solids concentration of 1500 mg/l (as in Reactors A and B) was diluted to

its original volume with supernatant from the original 1500 ml. This was used to charge Reactor C. An equal volume of sonicated material was again centrifuged, the supernate brought to its original volume as before and this was used to charge Reactor D. It should be made clear at this point, that at no time was concentrated material used to charge the various reactors. The amounts of enzyme, both soluble and particulate, were approximately the same as the concentrations found in the intact cells.

Chemicals and Analytical Procedures Used

The following chemicals were used in the experiments to determine the effect of return sludge disruption on rate of biological oxidation of specific components. These compounds were tested at two input concentrations and two sonication levels (S-1 and S-2 - see above).

1. LAS (Linear Alkylate Sulfonates) Reference LAS Acids, Soap and Detergent Manufacturers' Association. Lot 2-4, active LAS 4.11%. Prepared as 1000 mg/l stock solution in deionized water.
2. Ethylene glycol purified, City Chemical Corporation, New York. Ethylene-1,2-^{14}C glycol, ICN Chemical and Radioisotope Division, Irvine, California. Lot 5615-62 Sp. Activity 18.6 mC/mM. 100µc in 0.2 ml methanol. Cold ethylene glycol prepared as 1000 mg/l stock solution in deionized water.
3. NTA (Nitrilotriacetic Acid, Trisodium Salt) Lot No. 49B-1380, Sigma Chemical Co., St. Louis, Missouri. Prepared as 1000 mg/l (1.070 gm/l) stock solution in deionized water.
4. EDTA Ethylene Dinitrilotetraacetic Acid) Lot No. 30437 Baker Analyzed Reagent. Prepared as 1000 mg/l stock solution in deionized water (addition of sufficient 1N NaOH to dissolve prior to bringing to volume).
5. 2,4-Dichlorophenol, Eastman Organic Chemical 1933. Prepared as 1000 mg/l stock solution in deionized water. Adequate precaution should be used when handling this material.

SECTION V

EXPERIMENTAL RESULTS

The first consideration in the experimental phase was to define the conditions under which reproducible results, in terms of cell disruption, could be obtained.

Initial attempts with a flow through cell attached to the Biosonik III standard probe tip, gave erratic results. It was then decided to use a static system, with external cooling (salt-ice bath), in a container whose geometry would enhance the mixing due to cavitation. (See Figure 3.) This took the form of an aluminum funnel with the delivery spout bent back upon itself and sealed with solder. The funnel could hold 300 ml of concentrated sludge or other material. The probe tip when immersed in the funnel contents was approximately 1/2 in. from the side. The sonic energy was directed to the side, and then reflected at an angle, so that extremely vigorous mixing resulted. The funnel itself was supported on a piece of aluminum pipe, the whole of which (pipe plus funnel) was immersed in a salt-ice bath held in a Styrofoam^{R} bucket.

Survival curves were then generated with this apparatus to determine its effectiveness in disrupting the microorganisms in sludge, and in another system (suspension of Baker's yeast, approximately 3.4% dry weight). The results of typical survival experiments are shown in Figures 5 and 6. The longest disruption time used was for one hour. Two levels of sonication were then arbitrarily chosen to represent partially disrupted and completely disrupted cells, 20 minutes and 60 minutes, respectively. From Figure 5,

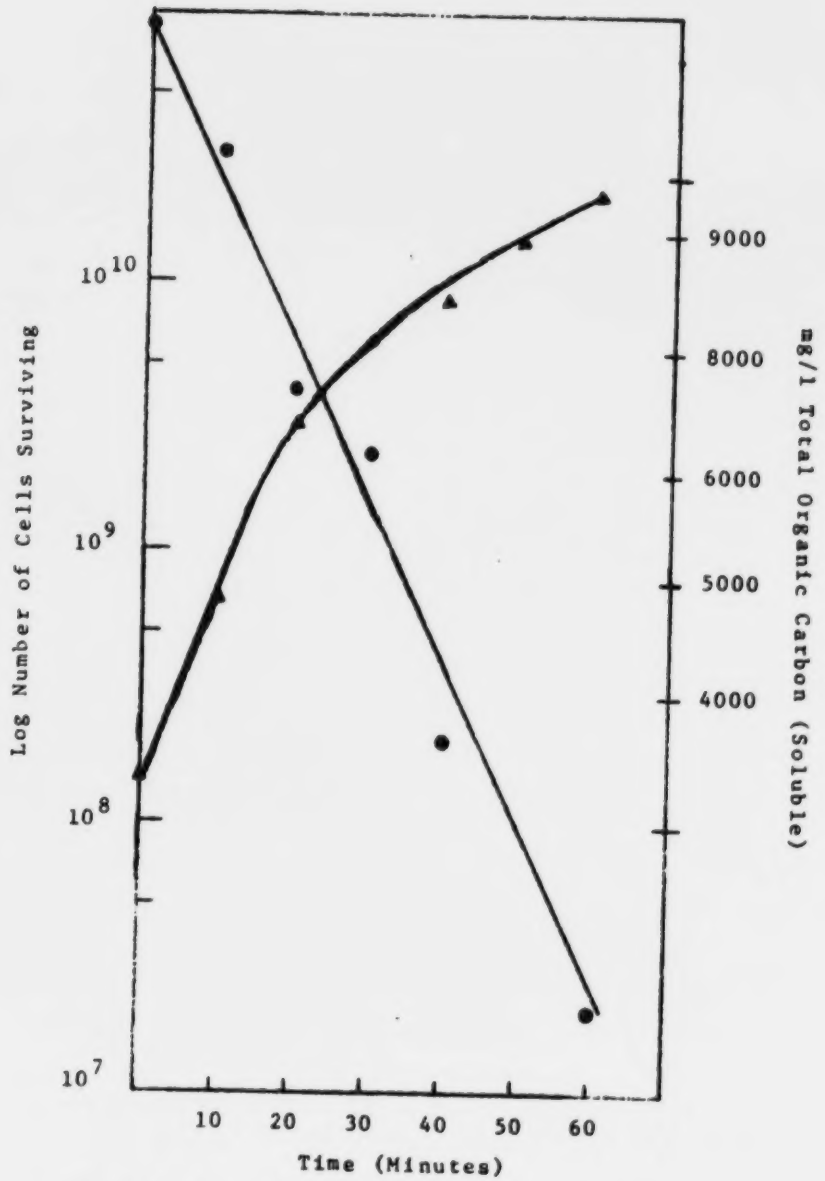

FIGURE 5. Cell Survival (as Most Probable Number) and Total Organic Carbon (Soluble) as a Function of Sonication Time. ● Log Cell Number ▲ Total Organic Carbon. Concentrated Sludge (5X). Bronwill Biosonik III Maximum Output 1 hr. Temperature 18C.

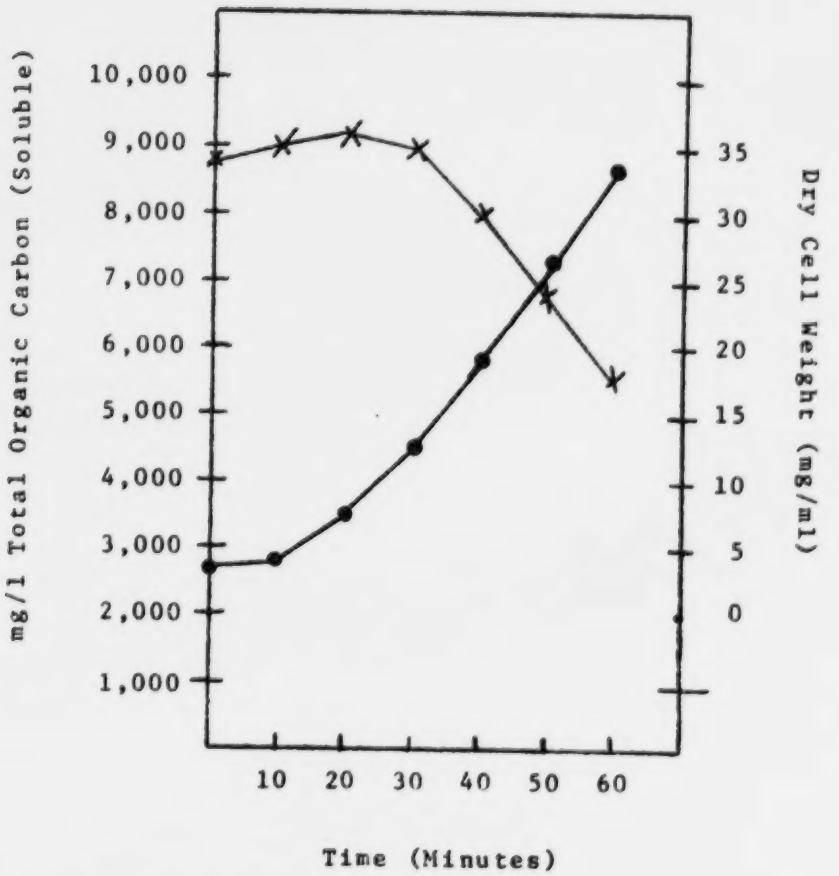

FIGURE 6. Soluble Organic Carbon and Dry Cell Weight Relationship in Baker's Yeast as a Function of Sonication Time. Bronwill Biosonik III Maximum Output.

● Organic Carbon
X Dry Cell Weight

it can be seen that the reduction in cell numbers by sonication in 20 minutes is 1.4 logs and the reduction in 60 minutes is approximately 3.4 logs.

The five experimental compounds were tested at two concentrations at each of the two sonication levels chosen. This was necessary to determine the effects of concentration and extent of cell disruption. Tables 1-9 represent the actual data generated in these experiments. It can be seen that for two of the compounds (Tables 7, 8, 9) EDTA, and ethylene glycol, a complete series of experiments was not attempted. This was because in the case of EDTA no satisfactory acclimated sludge was ever obtained (see Figure 19 and discussion). In the case of ethylene glycol the problem involved the use of the ^{14}C isotope form of the compound and the problems will be covered in detail under Activity.

Examination of the data in Tables 1-9 will demonstrate that the flow rates were not consistent throughout the course of the experimental work. Because of the small size of the wastewater inlets (see Figure 1) on the reactor, accumulations of suspended solids periodically caused variations in the input flow rates. These variations were compensated by manually increasing or decreasing the input flow to maintain an overall average of 5-6 ml per minute for all four reactors. This accumulation of solids occurred even though the wastewater used as carrier for the test compounds was filtered twice (1st through glass wool and then through Whatman #1 filter paper) to reduce the size of the particles remaining to a relatively constant value.

		S-1 3/1/72				S-2 3/8/72			
Reactor	Sample Number	Time (Min)	Test Compound (mg/l)	Solids (mg/l)	Volume Collected (ml)	Time (Min)	Test Compound (mg/l)	Solids (mg/l)	Volume Collected (ml)
A	0	0	2.5	1500	---	0	2.5	1850	---
	1	90	0.9	1200	590	90	0.4	1650	495
	2	180	0.5	900	540	180	<0.1	1300	540
	3	270	0.7	800	520	270	<0.1	1050	480
	4	360	0.4	540	555	360	<0.1	780	300
B	0	0	2.5	1500	---	0	2.5	2200	---
	1	90	0.9	1300	590	90	0.4	1740	520
	2	180	0.7	1110	510	180	<0.1	1270	500
	3	270	0.5	830	540	270	<0.1	1260	465
	4	360	0.3	570	540	360	<0.1	790	295
C	0	0	2.5	1500	---	0	2.5	1050	---
	1	90	0.9	1250	540	90	0.9	1310	500
	2	180	0.5	990	540	180	1.2	1060	500
	3	270	0.8	740	510	270	1.0	750	480
	4	360	0.6	570	470	360	1.0	460	250
D	0	0	2.5	400	---	0	2.5	600	---
	1	90	1.4	360	570	90	<0.1	594	530
	2	180	1.5	440	510	180	1.2	540	525
	3	270	1.4	255	555	270	0.9	387	470
	4	360	1.2	222	350	360	0.4	213	300

TABLE 1. Dichlorophenol Run
2.5 mg/l Initial and Input Concentration
S-1 (20 minutes sonication time)
S-2 (1 hour sonication time)

		S-1 2/28/72				S-2 3/6/72		
Reactor	Sample Number	Time (Min)	Test Compound (mg/1)	Solids (mg/1)	Volume Collected (ml)	Test Compound (mg/1)	Solids (mg/1)	Volume Collected (ml)
A	0	0	5.0	1900	---	5.0	2220	---
	1	90	2.4	1590	485	1.5	1700	480
	2	180	2.0	1060	500	1.5	1340	480
	3	270	1.5	870	550	0.6	1230	490
	4	360	1.1	565	730	0.5	950	495
B	0	0	5.0	1900	---	5.0	2150	---
	1	90	2.4	1510	630	1.8	1730	485
	2	180	2.1	1130	510	1.3	1360	490
	3	270	1.6	920	550	1.1	1100	495
	4	360	1.1	725	410	0.7	820	495
C	0	0	5.0	1500	---	5.0	2200	---
	1	90	2.6	1350	530	2.0	1450	485
	2	180	2.0	910	500	2.5	970	480
	3	270	2.1	900	500	2.1	740	490
	4	360	1.6	830	670	2.0	710	495
D	0	0	5.0	440	---	5.0	1000	---
	1	90	2.8	383	510	2.5	595	480
	2	180	2.6	280	550	2.3	512	485
	3	270	2.6	210	500	2.3	380	500
	4	360	2.1	408	750	2.0	328	505

TABLE 2. Dichlorophenol Run
5.0 mg/1 Initial and Input Concentration
S-1 (20 minute sonication time)
S-2 (1 hour sonication time)

		S-1 3/17/72					S-2 3/22/72				
Reactor	Sample No.	Time (Min)	Test Compound (mg/l)	Solids (mg/l)	Volume Collected (mg/l)	Total Organic Carbon (mg/l)	Settling Volume (ml)	Test Compound (mg/l)	Solids (mg/l)	Volume Collected (ml)	Total Organic Carbon (mg/l)	Settling Volume (ml)
A	0	0	5.0	1500	---	---		5.0	1800	---	---	
	1	90	2.9	1140	235	125	6.0	1.0	1480	485	197	7.0
	2	180	1.0	1030	730	143	6.0	0.5	1230	550	114	5.5
	3	270	1.4	750	520	128	4.0	0.05	880	540	119	4.0
	4	360	0.4	420	335	187	3.2	0.05	720	420	126	3.5
B	0	0	5.0	1960	---	---		5.0	1400	---	---	
	1	90	2.3	1560	305	159	6.0	1.0	1260	495	192	6.0
	2	180	0.4	1070	590	151	5.0	1.0	1170	485	206	4.5
	3	270	0.05	760	600	156	4.0	1.4	790	460	167	3.5
	4	360	0.05	280	360	235	3.0	0.05	660	510	181	3.0
C	0	0	5.0	1500	---	---		5.0	900	---	---	
	1	90	0.05	1070	175	336	0.5	1.8	710	495	497	<0.5
	2	180	1.60	713	765	226	0.5	1.4	513	380	414	<0.5
	3	270	1.85	560	560	265	0.5	2.1	497	450	340	<0.5
	4	360	1.20	400	345	271	0.5	1.6	360	260	286	<0.5
D	0	0	5.0	600	---	---		5.0	154	---	---	
	1	90	2.7	313	100	382	<0.5	1.6	141	465	359	<0.5
	2	180	1.4	114	890	296	<0.5	3.1	132	525	312	<0.5
	3	210	1.4	97.5	515	283	<0.5	2.5	127	510	369	<0.5
	4	360	0.5	60	360	283	<0.5	3.4	114	220	234	<0.5

TABLE 3. Linear Alkylate Sulfonate Run (LAS)
5.0 mg/l Initial and Input Concentrations
S-1 (20 minute sonication time)
S-2 (1 hour sonication time)

		S-1 3/15/72				S-2 3/20/72		
Reactor	Sample Number	Time (Min)	Test Compound (mg/1)	Solids (mg/1)	Volume Collected (ml)	Test Compound (mg/1)	Solids (mg/1)	Volume Collected (ml)
A	0	0	10.00	2000	---	10.00	1500	---
	1	90	2.30	2050	490	1.60	1260	500
	2	180	2.30	1180	505	4.40	1210	520
	3	270	0.45	870	740	3.80	1000	505
	4	360	0.50	430	310	3.50	880	490
B	0	0	10.00	2000	---	10.00	1600	---
	1	90	2.10	1510	540	4.40	1280	500
	2	180	0.75	1110	520	3.80	1390	520
	3	270	1.00	890	780	4.20	980	480
	4	360	2.30	380	310	4.20	620	485
C	0	0	10.00	1800	---	10.00	800	---
	1	90	7.05	1330	530	4.40	584	460
	2	180	1.40	1240	530	6.60	636	495
	3	270	1.80	593	560	3.80	443	490
	4	360	2.50	380	360	3.50	218	490
D	0	0	10.00	500	---	10.00	150	---
	1	90	2.70	376	510	7.10	109	485
	2	180	5.30	260	490	5.30	70	525
	3	270	2.70	143	520	3.40	112	470
	4	360	3.60	84	450	2.05	45	490

TABLE 4. Linear Alkylate Sulfonate
10.0 mg/1 Initial and Input Concentrations
S-1 (20 minute sonication time)
S-2 (1 hour sonication time)

		S-1 5/23/72				S-2 5/29/72			
Reactor	Sample Number	Time (Min)	Test Compound (mg/l)	Solids (mg/l)	Volume Collected (ml)	Time (Min)	Test Compound (mg/l)	Solids (mg/l)	Volume Collected (ml)
A	0	0	100.0	1500	---	0	100.0	1100	---
	1	90	73.5	1260	640	90	65.0	970	450
	2	180	53.3	800	360	180	36.0	850	470
	3	270	50.0	870	470	270	46.0	740	450
	4	360	36.4	740	540	360	30.0	620	560
B	0	0	100.0	1500	---	0	100.0	1100	---
	1	90	78.4	1110	490	90	58.0	1040	470
	2	180	59.0	830	460	180	61.0	930	470
	3	270	52.0	650	480	270	42.0	910	460
	4	360	57.0	810	490	360	26.0	780	490
C	0	0	100.0	1500	---	0	100.0	460	---
	1	90	98.6	1040	460	90	81.0	400	490
	2	180	70.3	610	470	180	69.0	355	490
	3	270	61.4	450	495	270	57.0	360	490
	4	360	52.0	540	490	360	61.0	408	470
D	0	0	100.0	130	---	0	100.0	64.0	---
	1	90	85.0	102	540	90	69.0	62.0	540
	2	180	67.1	81	550	180	65.0	85.0	490
	3	270	48.5	57	350	270	44.0	59.0	530
	4	360	47.0	69	490	360	42.0	55.0	360

TABLE 5. Nitrilotriacetic Acid Run
100 mg/l Initial and Input Concentrations
S-1 (20 minute sonication time)
S-2 (1 hour sonication time)

		S-1 4/26/72				S-2 4/28/72		
Reactor	Sample Number	Time (Min)	Test Compound (mg/l)	Solids (mg/l)	Volume Collected (ml)	Test Compound (mg/l)	Solids (mg/l)	Volume Collected (ml)
A	0	0	150	1500	---			
	1	90	134	1480	440			
	2	180	124	1230	440			
	3	270	126	1045	585			
	4	360	126	820	530			
B	0	0	150	1500	---	150	1000	---
	1	90	131	1360	450	126	790	440
	2	180	132	1260	440	122	680	580
	3	270	122	950	560	87.2	420	525
	4	360	124	880	525	46.0	410	460
C	0	0	150	1500	---	150	292	---
	1	90	195	1250	480	141	264	500
	2	180	179	1000	480	129	232	580
	3	270	137	880	545	126	156	475
	4	360	126	640	500	58.2	188	525
D	0	0	150	191	---	150	135	---
	1	90	155	187	500	145	50	520
	2	180	140	168	520	136	54	490
	3	270	126	123	560	127	108	575
	4	360	124	81	610	122	42	475

TABLE 6. Nitrilotriacetic Acid Run
150 mg/l Initial and Input Concentrations
S-1 (20 minute sonciation time)
S-2 (1 hour sonication time)

Reactor	Sample Number	Time (Min)	Test Compound (mg/l)	Solids (mg/l)	Volume Collected (ml)
A	0	0	150		
	1	90	128		
	2	180	140		
	3	270	146		
	4	360	142		
B	0	0	150		
	1	90	122		
	2	180	138		
	3	270	140		
	4	360	146		
C	0	0	150		
	1	90	126		
	2	180	156		
	3	270	174		
	4	360	158		
D	0	0	150		
	1	90	136		
	2	180	132		
	3	270	138		
	4	360	146		

TABLE 7. Ethylenedinitrilotriacetic Acid (EDTA) Run 5/2/72 150 mg/l S-1

Reactor	Sample Number	Time (Min)	Test Compound (cpm)	Solids (mg/l)	Volume Collected (ml)
A	0	0	530	1700	---
	1	15	---	1660	190
	2	90	499	1360	300
	3	180	444	1020	715
	4	270	465	800	625
		360	412	650	560
B	0	0	520	1560	---
	1	15	---	1520	500
	2	90	530	1380	140
	3	180	385	1200	530
	4	270	400	1000	205
		360	470	700	420
C	0	0	580	1300	---
	1	15	---	1300	210
	2	90	470	1140	260
	3	180	550	620	660
	4	270	530	800	500
		360	600	730	510
D	0	0	550	80	---
	1	15	---	80	210
	2	90	620	40	260
	3	180	600	55	590
	4	270	550	65	410
		360	530	63	610
E Batch		0	334	---	---
		180	140	---	---
		360	86	---	---

TABLE 8. Ethylene-1,2-^{14}C-glycol Run 1/25/72
10 mg/l S-1 (20 minute sonication time)

Reactor	Sample Number	Time (Min)	Test Compound (cpm)	Solids (mg/l)	Volume Collected (ml)
A	0	0	550	1500	---
	1	90	490	1390	310
	2	180	440	1190	180
	3	270	370	1200	340
	4	360	570	890	295
B	0	0	550	1500	---
	1	90	500	1340	305
	2	180	470	1080	215
	3	270	430	500	355
	4	360	430	671	330
C	0	0	590	940	---
	1	90	530	820	500
	2	180	580	720	62
	3	270	545	380	305
	4	360	340	320	300
D	0	0	530	280	---
	1	90	600	210	330
	2	180	555	250	120
	3	270	560	106	320
	4	360	570	143	310
E Batch Run	0	0	490	1260	---
	1	90	350		---
	2	180	200	1270	---
	3	270	175		---
	4	360	180	1110	---

TABLE 9. Ethylene-1,2-^{14}C-glycol 1/26/72
(Extended Retention Time) S-1
(20 minute sonication time)
10 mg/l

Input flow was controlled to a great extent by using a Masterflex peristaltic pump Model 7104 (Cole-Parmer Co., Chicago, Illinois), the wastewater carrier plus test compound was fed to a constant head reservoir on the reactor, and then distributed through a manifold to the individual reactors, and controlled manually as described above.

Aeration to the four reactors was through a manifold and then to each reactor through a glass, fritted-disc sparger. Initially it was considered that aeration alone would provide sufficient turbulence to keep the reactors well-mixed. However, preliminary experiments demonstrated that the mixing provided by aeration was not sufficient to keep the solids in suspension, consequently, solids settled in the corners of the reactors. An attempt to use magnetic mixing bars to alleviate the problem was not successful. The problem was solved by using double bladed aluminum paint mixers, held in place in a Lucite^{R} sleeve and belt driven by a small electric motor whose speed was controlled by a powerstat. (See Figure 1.)

Samples were taken and analyzed for the various parameters at 90 minute intervals. To retard further degradation by microorganisms 1 ml of 10% (w/v) Sodium azide was added to each collection beaker. Samples, if not analyzed immediately, were held at 4C until analysis could be completed.

It can readily be seen from the data that no really direct comparison between different experiments can be made. The only direct comparisons which can be made are between the various

reactors within any given experiment. The reason for this is that the experiments were performed at different times, often a month or more apart and necessarily with a different sludge, acclimated at a different time. However, the conditions and operating characteristics of the reactors and disruption procedures were sufficiently reproducible to enable some comparative generalizations to be made both inter and intra experiment.

Analysis of Results

Mathematical analysis of a continuous culture system or a homogeneous, single stage stirred fermenter, (with aeration) (Herbert, 1961) must take into account the flow characteristics of the reactor system and the kinetics of the microbial growth process.

The washout curve for adequately mixed reactors of this type is described by the equation:

$$C/C_o = e^{-Dt}$$

where: C_o = initial concentration of substance in the reactor

C = concentration of substance of any time t

D = dilution rate

t = time of sampling

The dilution rate D may be calculated from the flow rate f and the volume of the reactor v as $D = f/v$ or flow per unit volume.

The inverse of the dilution rate $1/D$ or v/f is equal to $\bar{t}$ the mean retention time. So that a plot of C/C_o versus $t/\bar{t}$ will

give a curve which describes the concentration of solids being washed out of the reactor, and comparison of this curve with the theoretical curve $C/C_o = e^{-Dt}$ will show the adequacy of mixing in the reactor.

The concentration of substrate in the effluent from the reactor describes the concentration in the reactor at any given time and thus analyses can be made of the amount of substrate removed as a function of time (removal rate) or as a function of the amount of solids present or "activity", which we define operationally as $\frac{\text{mg of substrate removed}}{\text{mg of solids added.}}$.

Since we are starting with a concentration of substrate in the reactor which is equivalent to the concentration of the substrate in the carrier waste water then if no reaction or chemical transformation is occurring in the reactor (i.e., substrate removal)

Input = Output.

If, however, the substrate is being removed then:

Amount of Substrate Removed = Input-Output.

Using these relationships, we can now turn to a discussion of the experimental results in terms of mixing, "activity", removal rate, enzymes, total organic carbon relationships and future research needed.

Mixing Characteristics - Total Suspended Solids

Using the relationships previously described for washout curves, one can examine the washout of total suspended solids from each of the four reactors to determine whether adequate mixing was maintained. This was especially important in the study of the disrupted cells and the supernatant fraction from the disrupted cells. Obviously microorganism, enzymes or catalysts of any other nature cannot accomplish a chemical transformation unless they can come into contact with the appropriate substrate. This is especially true for enzymatic reactions because of the specificity of enzyme for substrate which is one of the unique characteristics of such reactions. Tenny and Budzin (1972) have recently shown the results of inadequate mixing in anaerobic digesters. It can be seen from Figures 7-18 that in most instances the mixing of the four reactors was, at a minimum, adequate. The shape of the experimental washout curves, except in a few instances (Figures 8C and D, 11D, 16D, 17D, 21D) follow the theoretical washout curves generated for that reactor in a particular experiment. Most of the variation is seen in reactor D and this might be expected since in all cases the concentration of suspended solids in Reactor D is the lowest of the four reactors and in some cases approaches the solids input of the waste water used as substrate carrier.

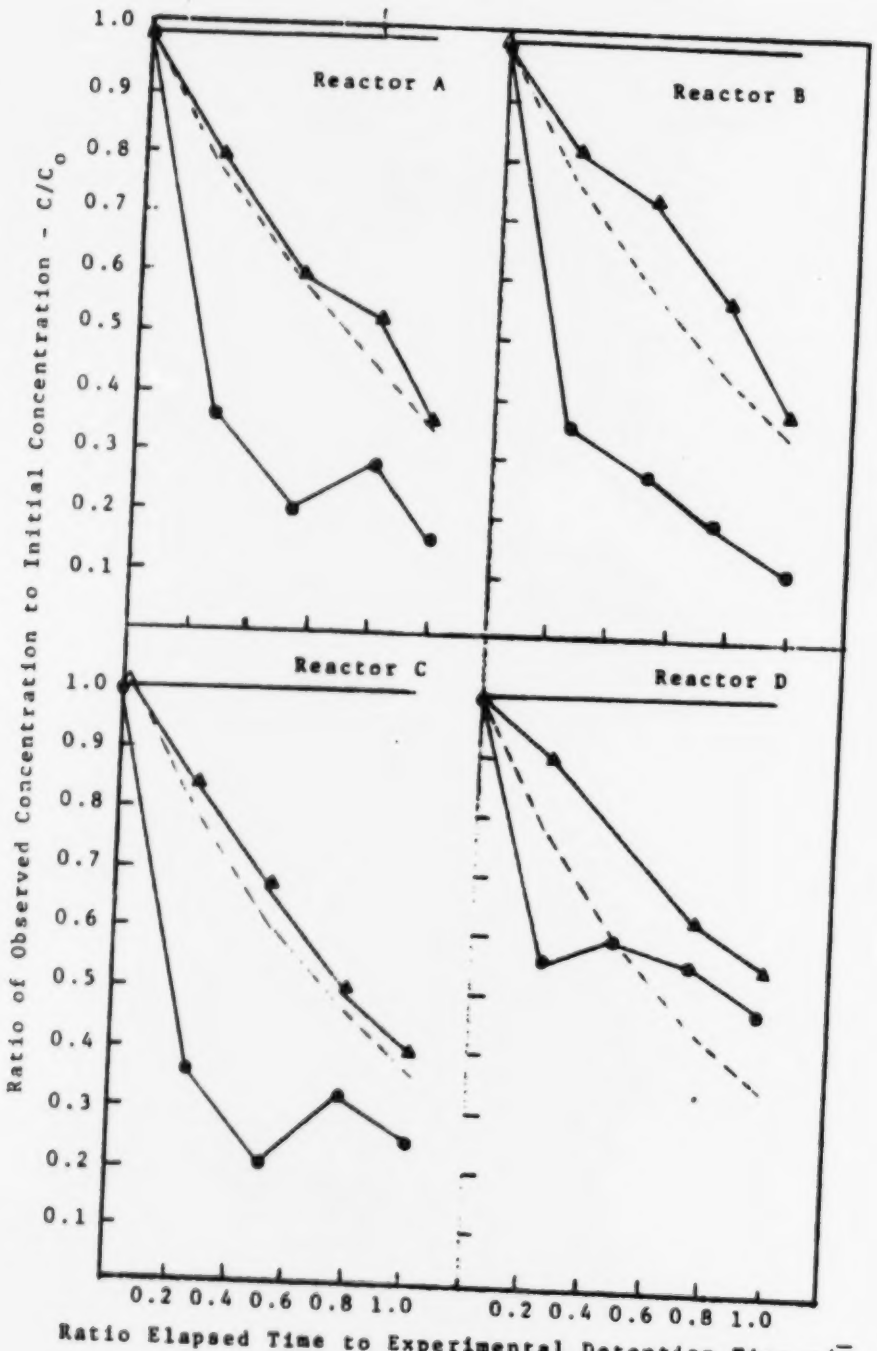

FIGURE 7. Dichlorophenol (DCP) Run, 2,5 mg/1, S-1

Dichlorophenol —— Theoretical • Experimental
Total Suspended Solids --- Theoretical ▲ Experimental

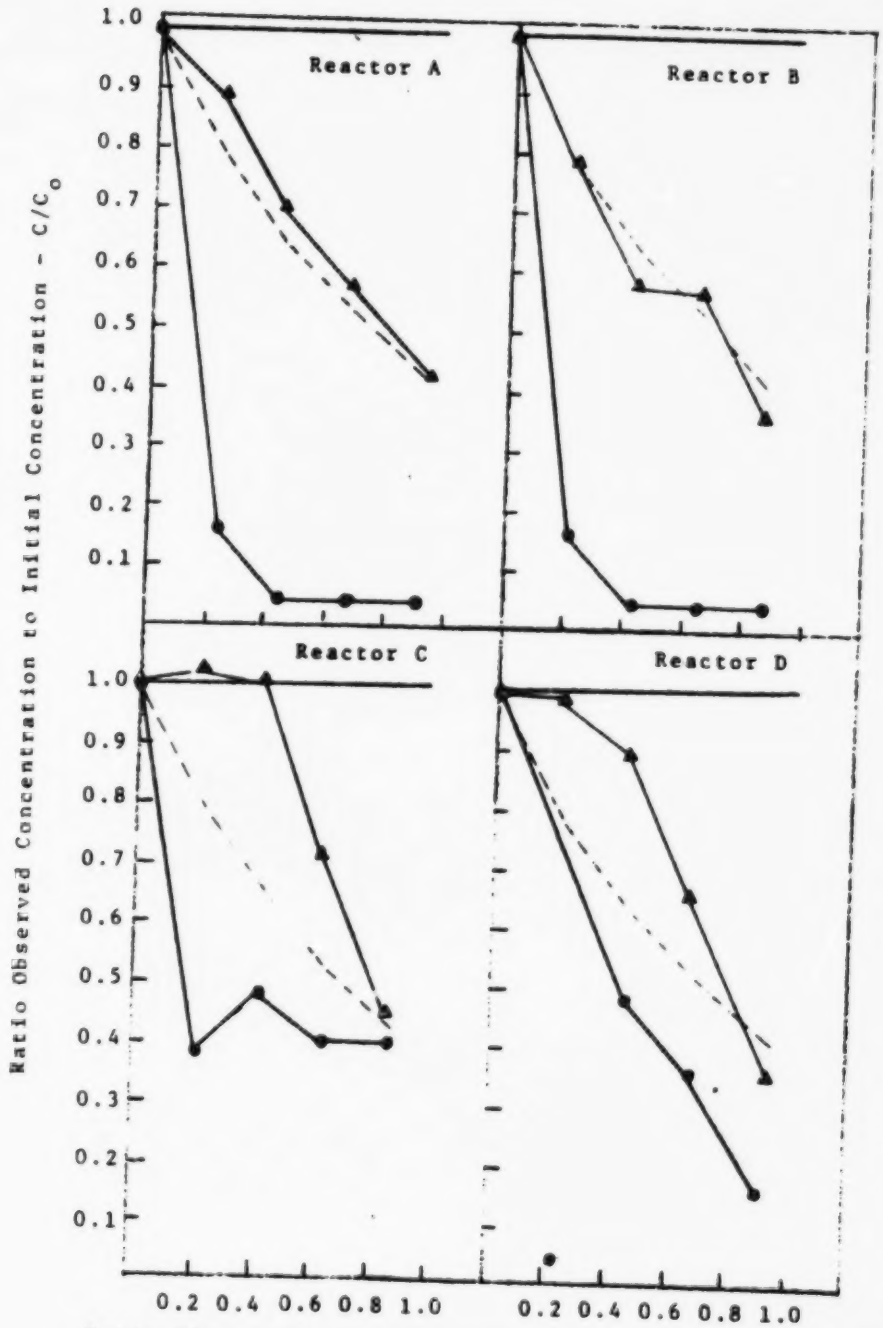

FIGURE 8. Dichlorophenol (DCP) Run 2.5 mg/l S-2

Dichlorophenol —— Theoretical • Experimental
Total Suspended Solids ----- Theoretical ▲ Experimental

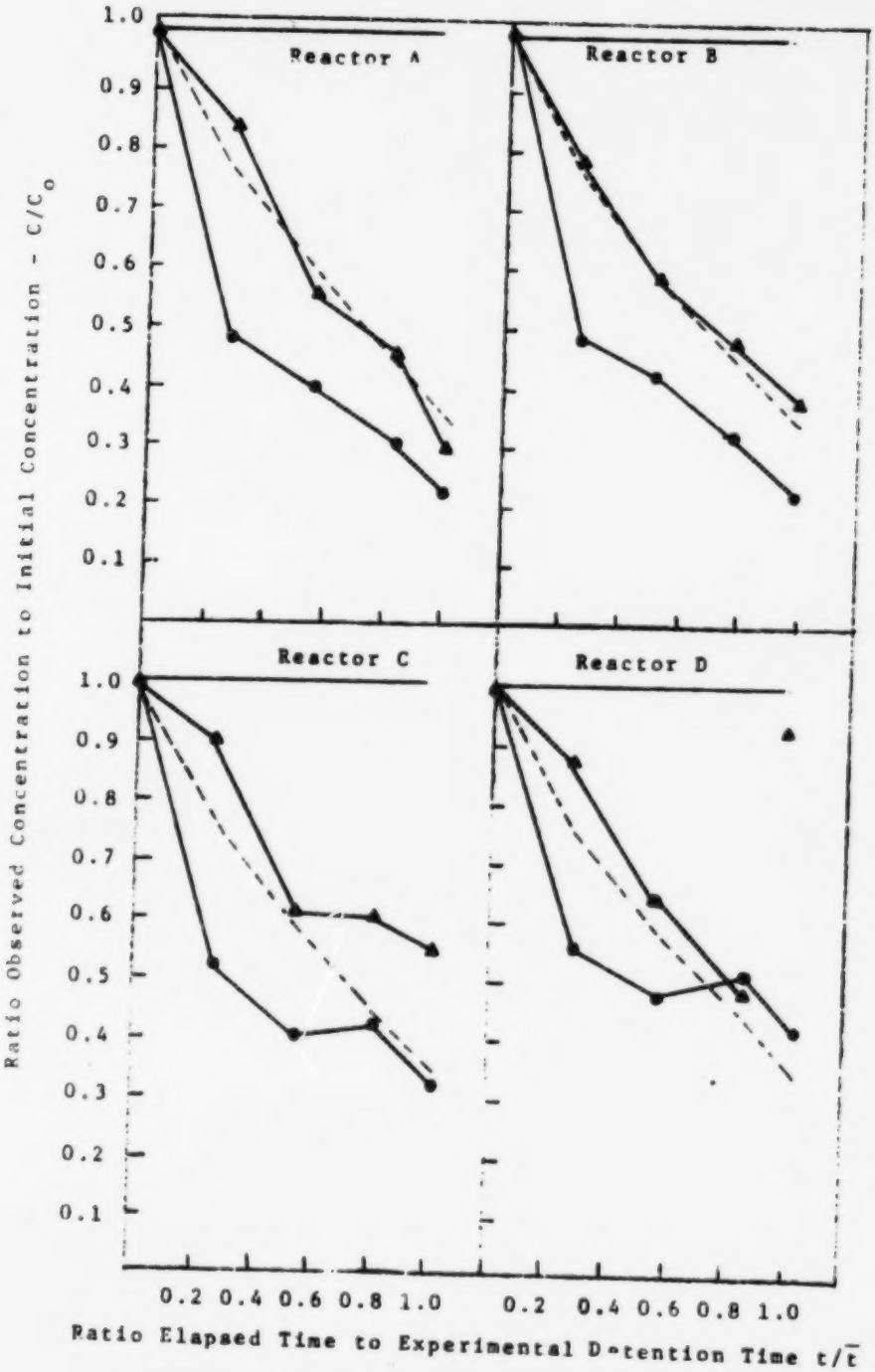

FIGURE 9. Dichlorophenol (DCP) Run 5.0, S-1

Dichlorophenol
Total Suspended Solids —— Theoretical • Experimental
--- Theoretical ▲ Experimental

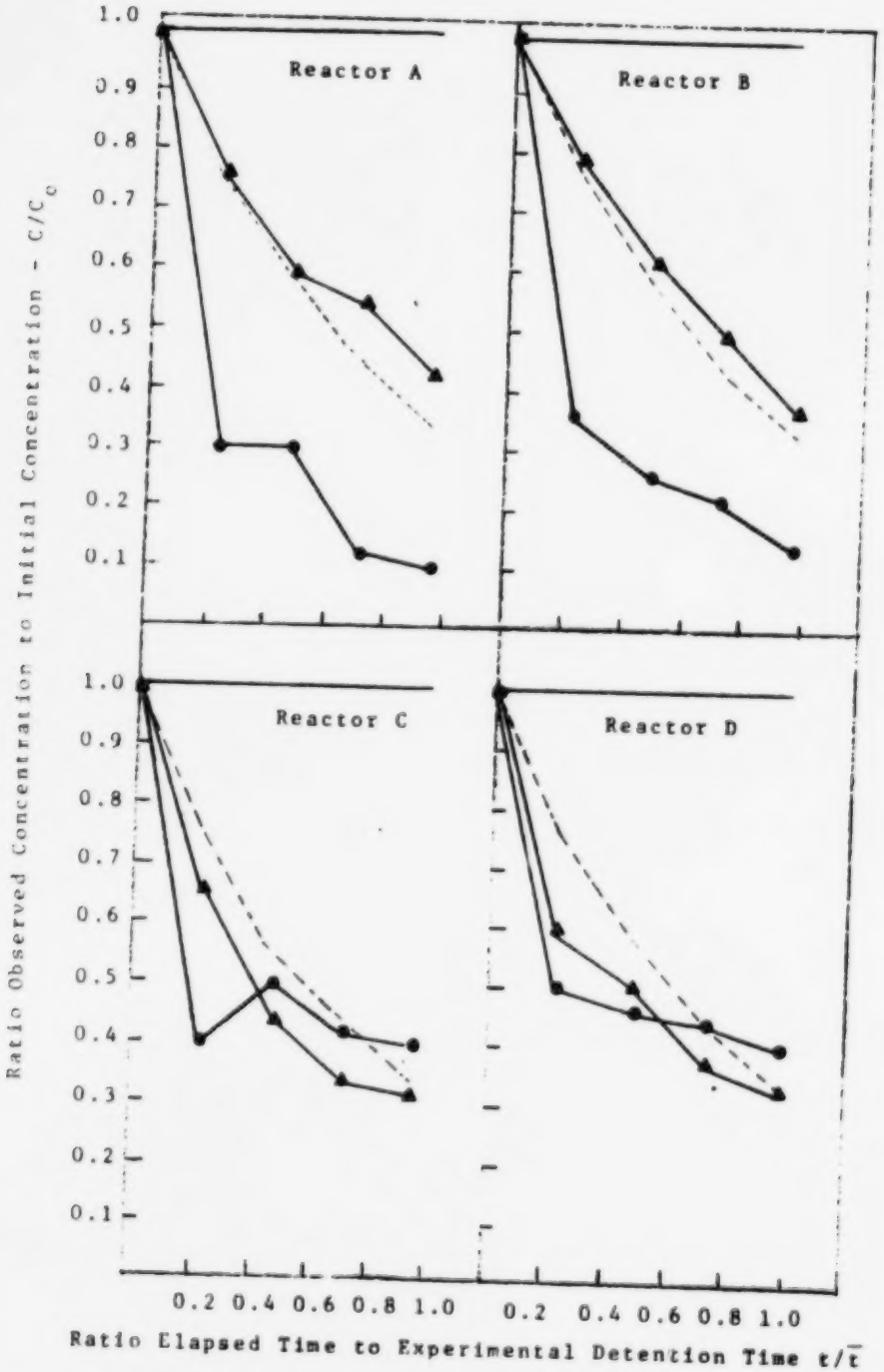

FIGURE 10. Dichlorophenol (DCP) Run 5.0 mg/1, S-2

Dichlorophenol —— Theoretical ● Experimental
Total Suspended Solids --- Theoretical ▲ Experimental

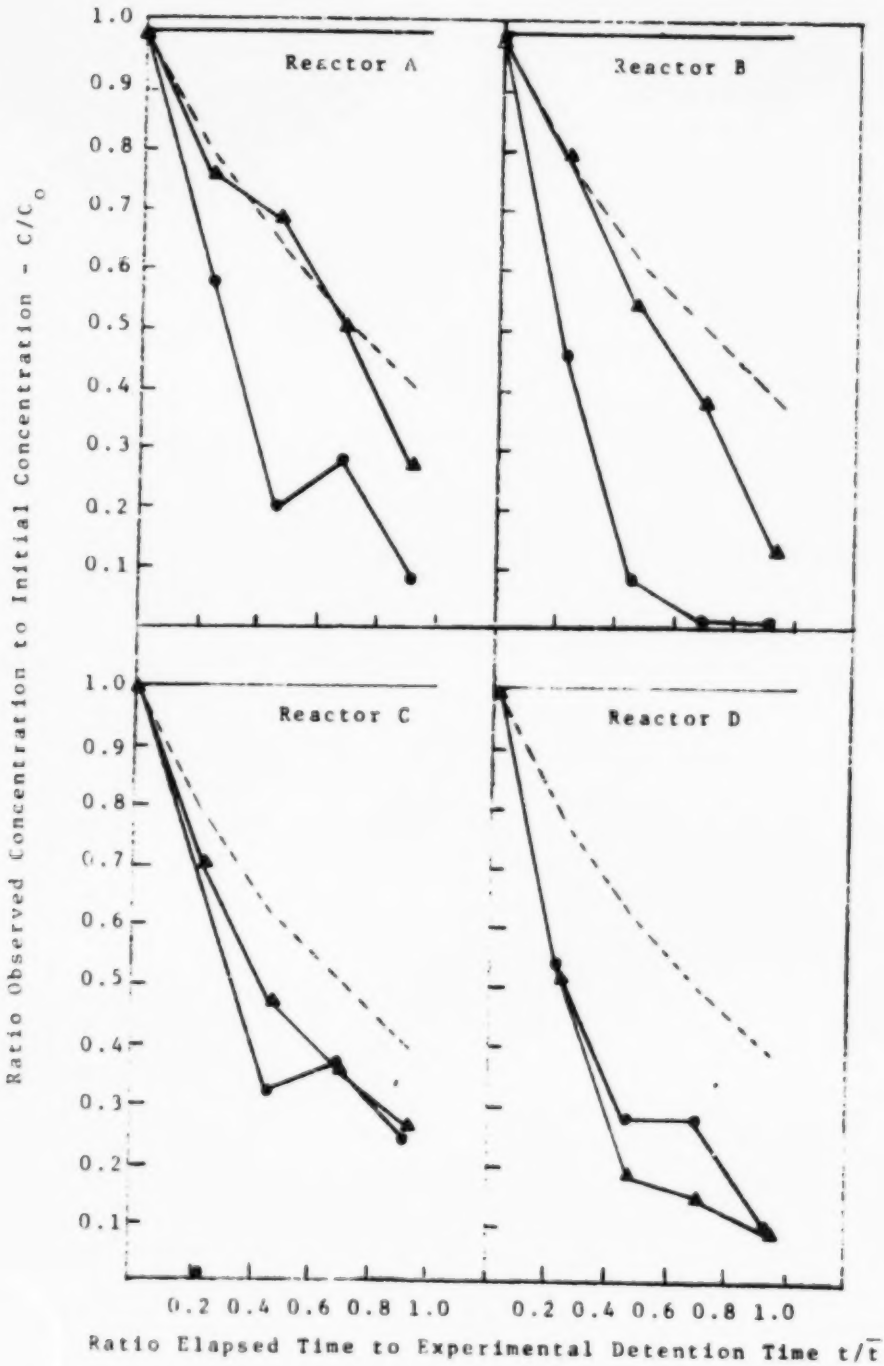

FIGURE 11. Linear Alkylate Sulfonate (LAS) Run, 5.0 mg/l, S-1

LAS Concentration —— Theoretical • Experimental
Total Suspended Solids --- Theoretical ▲ Experimental

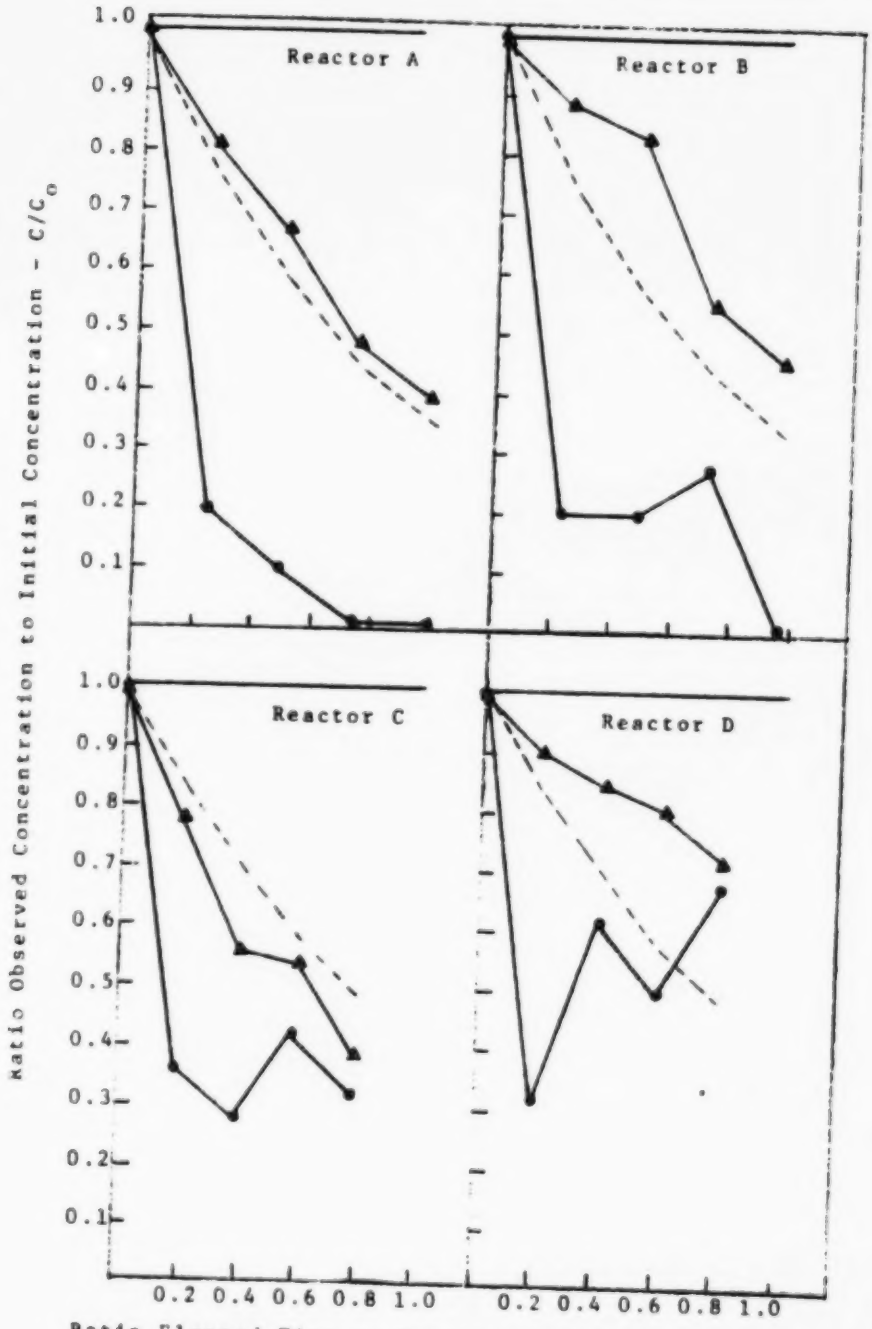

FIGURE 12. Linear Alkylate Sulfonate (LAS) Run, 5.0 mg/l, S-2

LAS Concentration —— Theoretical • Experimental
Total Suspended Solids --- Theoretical ▲ Experimental

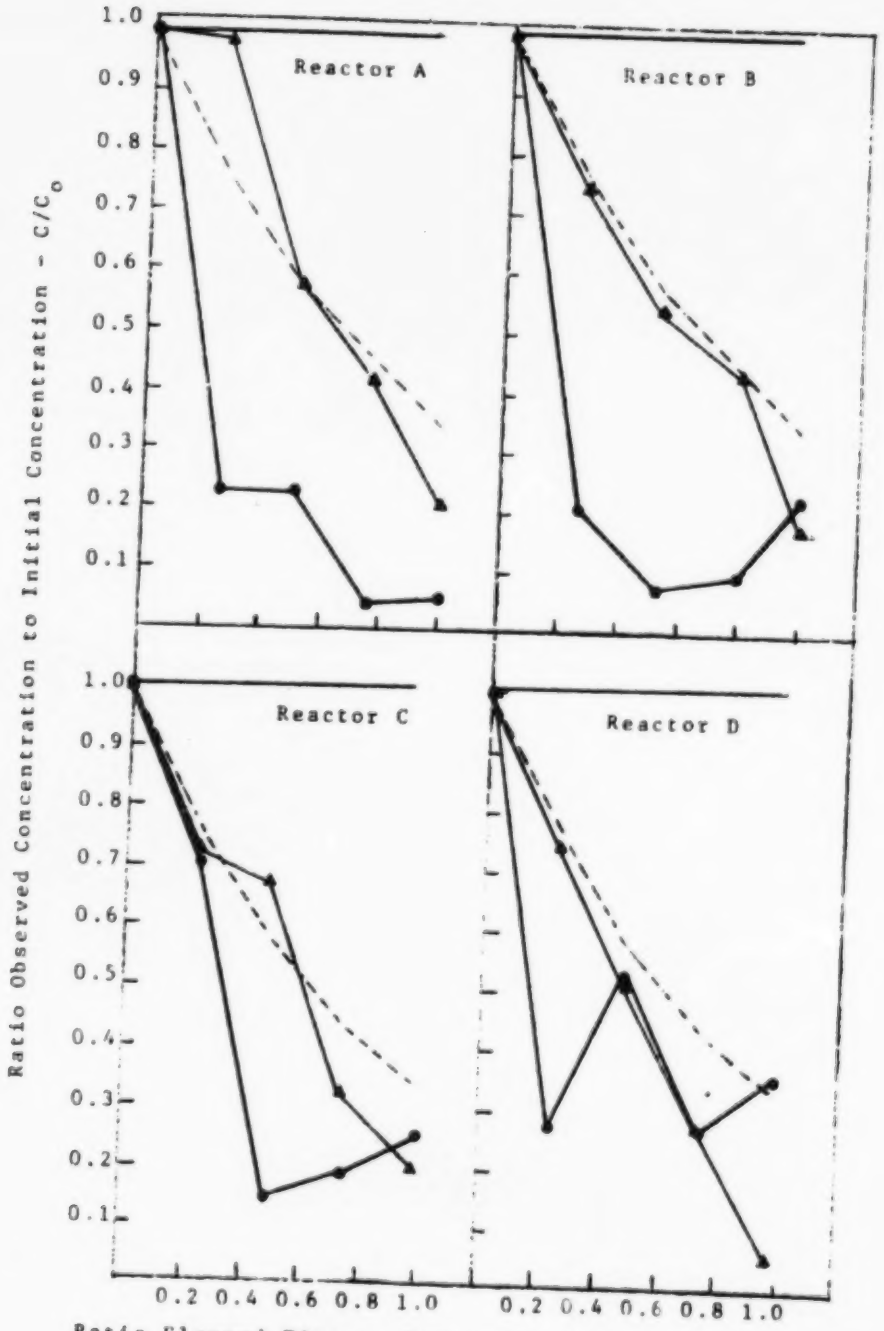

FIGURE 13. Linear Alkylate Sulfonate (LAS) Run, 10 mg/l, S-1

LAS Concentration —— Theoretical • Experimental
Total Suspended Solids --- Theoretical ▲ Experimental

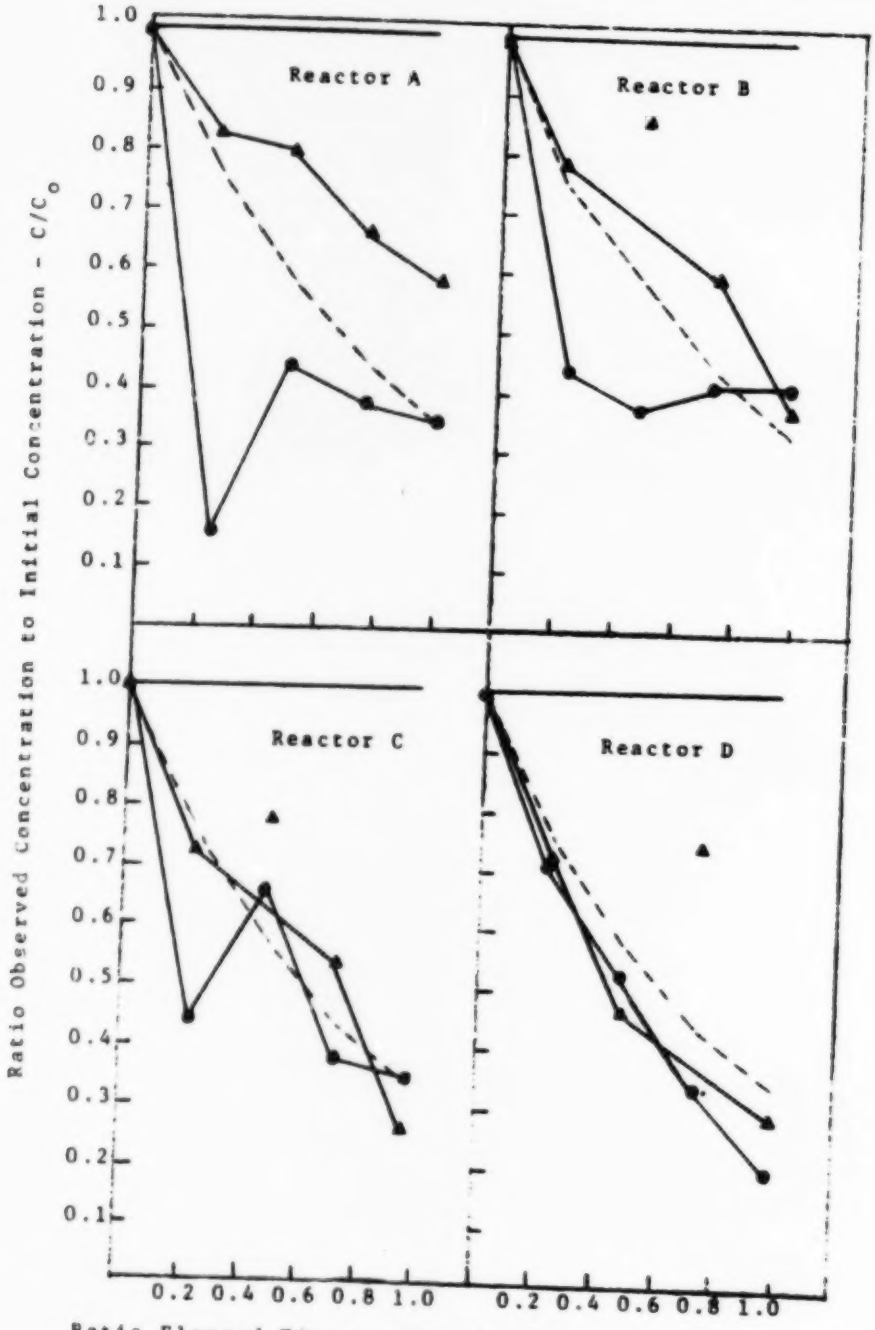

FIGURE 14. Linear Alkylate Sulfonate (LAS) Run, 10 mg/l, S-2

LAS Concentration —— Theoretical ● Experimental
Total Suspended Solids --- Theoretical ▲ Experimental

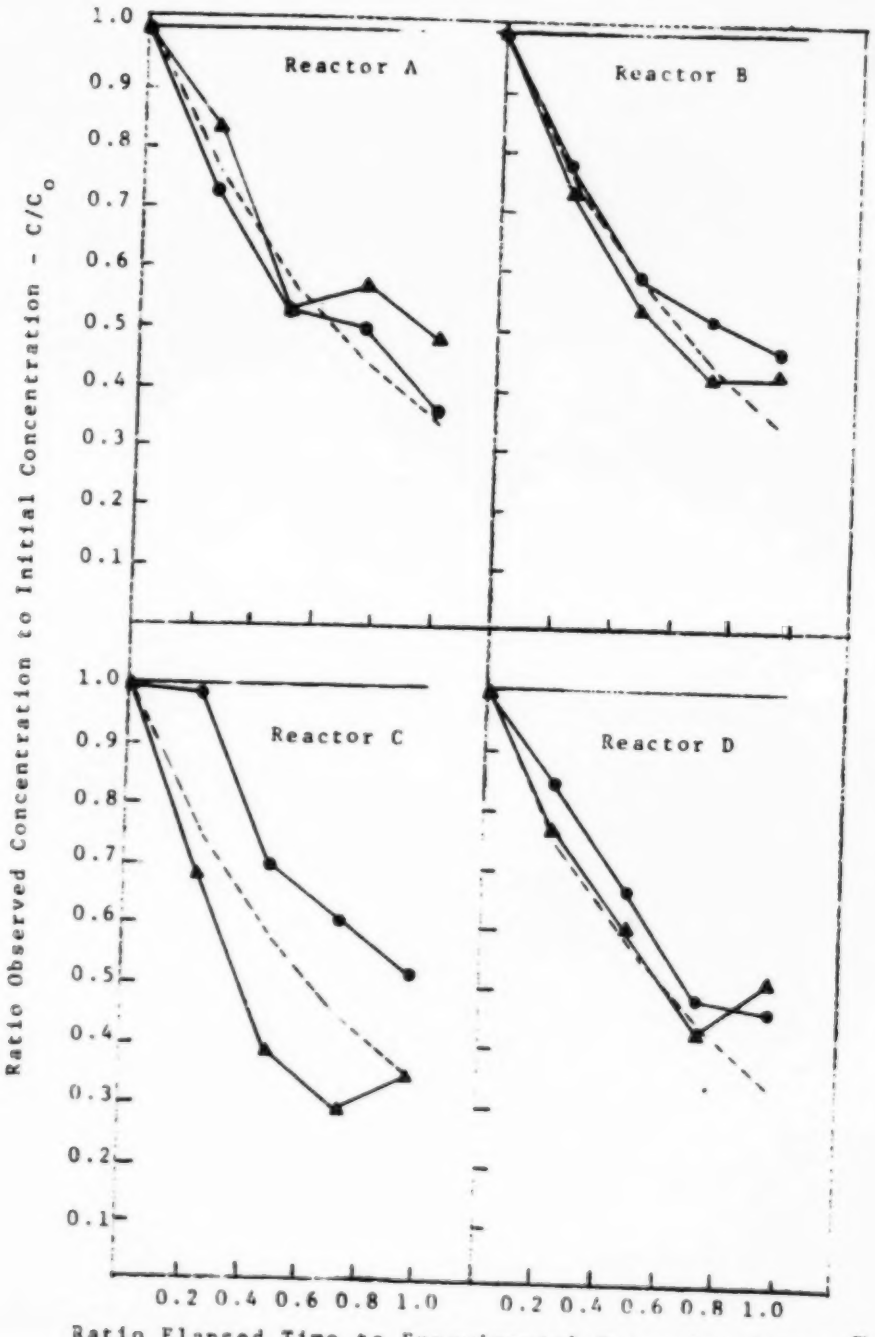

FIGURE 15. Nitrilotriacetic Acid (NTA) Run, 100 mg/l, S-1

NTA Concentration —— Theoretical • Experimental
Total Suspended Solids --- Theoretical ▲ Experimental

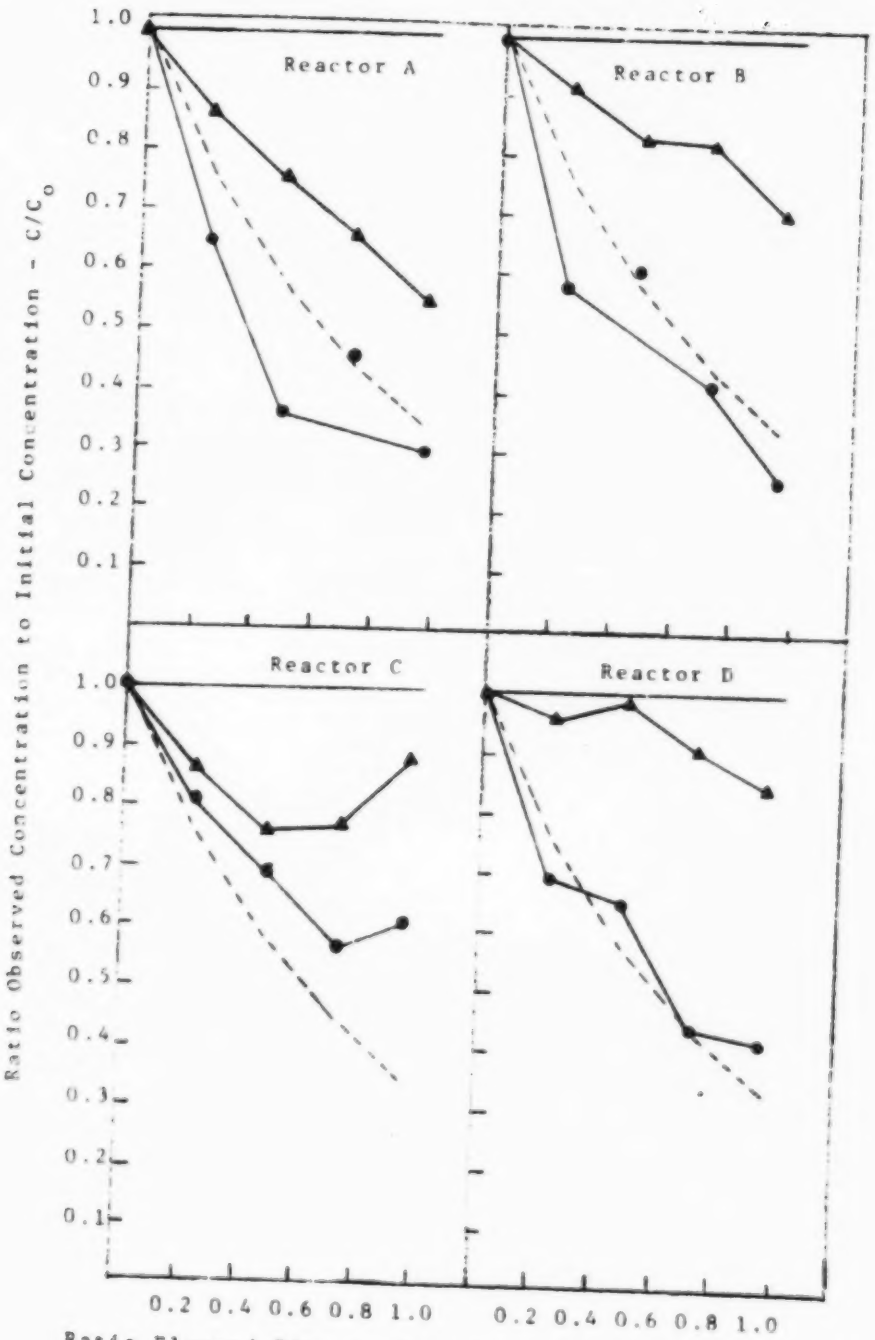

FIGURE 16. Nitrilotriacetic Acid (NTA) Run, 100 mg/l, S-2

NTA Concentration —— Theoretical ● Experimental
Total Suspended Solids --- Theoretical ▲ Experimental

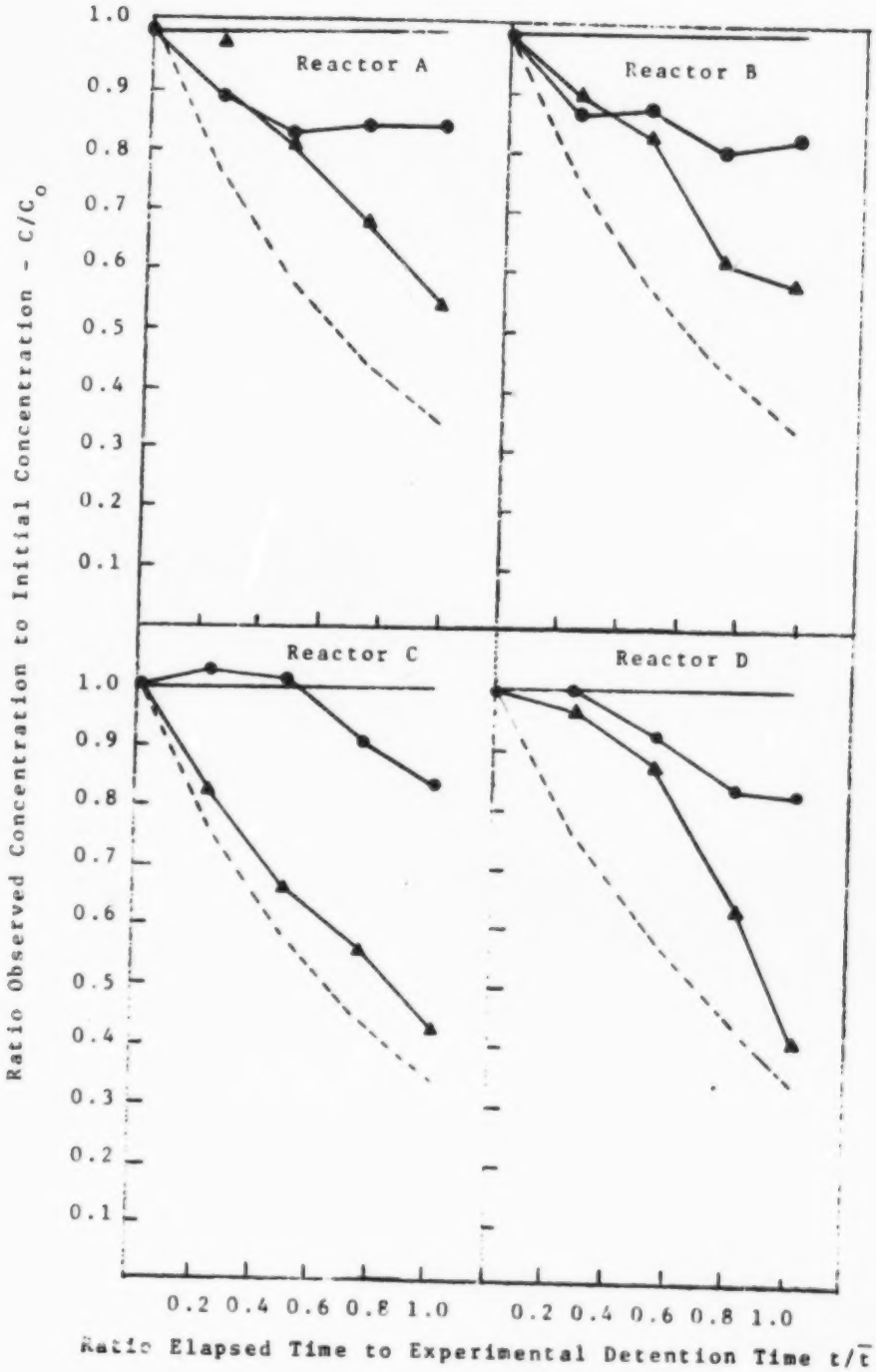

FIGURE 17. Nitrilotriacetic Acid (NTA) Run, 150 mg/1, S-1

NTA Concentration —— Theoretical ● Experimental
Total Suspended Solids --- Theoretical ▲ Experimental

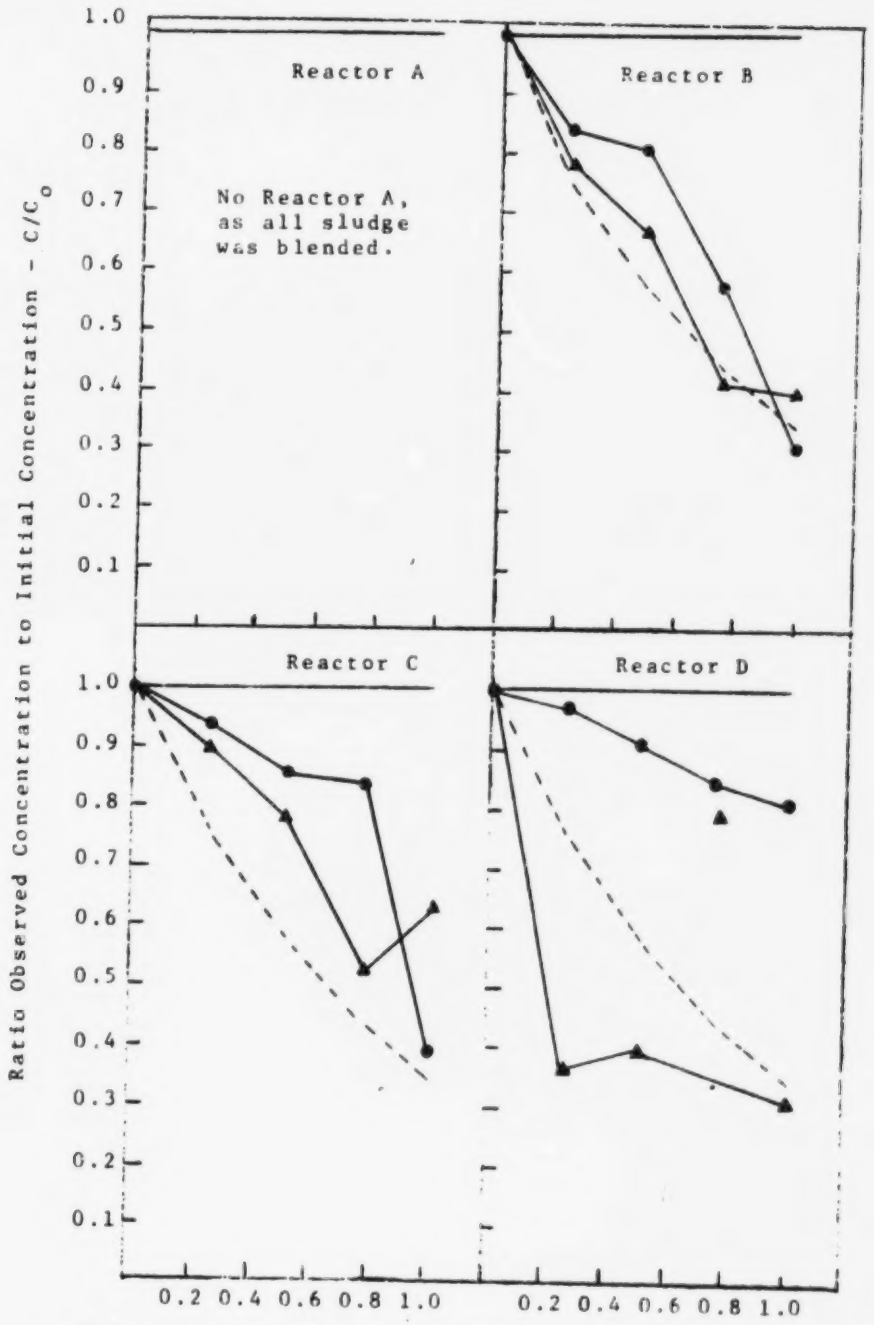

FIGURE 18. Nitrilotriacetic Acid (NTA) Run, 150 mg/l, S-2

NTA Concentration —— Theoretical • Experimental
Total Suspended Solids --- Theoretical ▲ Experimental

"Activity"

We have operationally defined "activity" as the ratio of amount of substrate removed (in milligrams) to the amount of solids added (in milligrams). This "activity" calculation allows us to compare, on the basis of suspended solids only, the effectiveness of test compound removal by each test condition.

Dichlorophenol (DCP)

Tables 10 and 11 show the "activity" data for Dichlorophenol at two concentrations, 2.5 mg/l and 5.0 mg/l at sonication levels of 20 minutes (S-1) and 1 hour (S-2). It can be seen that, in each instance, the "activity" in Reactor D is at least two times greater than the activity in Reactors, A, B, C. This implies that there is a considerable amount of adsorption onto particulate disrupted material or degradation of enzymes in Reactor C. Adsorption of enzymes onto particulates probably results in the inactivation of the enzymes due to masking of the active sites. Even so, the "activity" of Reactor C is equivalent to that in Reactors A and B which contain undisrupted microorganisms, even though C contains a minimum of 15X less cells (S-1) to a maximum of 1000X less cells (S-2). Dichlorophenol is probably converted to 4-chlorocatechol degraded through the β-keto adipate pathway (Evans and Moss, 1951). The relationship of the enzymes involved to the cell membrane will be discussed in a later section.

	Reactor	mg Test Compound Removed	mg Solids Added	"Activity" ($x10^{-3}$)
S-1 3/1/72	A	8.32	3000	3.0
	B	8.45	3000	3.0
	C	7.50	3000	3.0
	D	4.80	800	6.0
S-2 3/8/72	A	9.01	3700	2.0
	B	8.91	4400	2.0
	C	5.55	2100	3.0
	D	7.74	1200	6.0

TABLE 10. Dichlorophenol Runs. 2.5 mg/l at Two Sonication Levels

$$\text{Activity} = \frac{\text{mg test compound removed}}{\text{mg solids added.}}$$

	Reactor	mg Test Compound Removed	mg Solids Added	"Activity" ($x10^{-3}$)
S-1 2/28/72	A	15.34	3800	4.0
	B	14.39	3900	4.0
	C	13.30	3000	4.0
	D	11.61	880	13.0
S-2 3/6/72	A	16.75	4440	4.0
	B	16.03	4300	4.0
	C	11.56	4400	4.0
	D	11.42	2000	6.0

TABLE 11. Dichlorophenol Runs. 5.0 mg/l at Two Sonication Levels

$$\text{Activity} = \frac{\text{mg test compounds removed}}{\text{mg solids added}}$$

Linear Alkylate Sulfonate (LAS)

There is again no significant difference in the calculated "activity" of the sludge acclimated to LAS in Reactors A, B and C (Tables 12 and 13) at either concentration or sonication level (the twofold increase seen in Reactor C, Table 13, may be an artifact. The startling result here is the "activity" associated with Reactor D in all cases for LAS. The "activity" is from 3-10 times greater, which implies that this enzyme is not particle bound but free in the cytoplasm and that adsorption inhibition and degradation might conceivably be minimized by lowering the solids concentration in the treatment reactor.

Nitrilotriacetic Acid (NTA)

Tables 14 and 15 show the "activity" data for NTA. There seems to be a discrepancy with respect to the increase in "activity" of Reactor D in Table 14, to that in Table 15. The reason for this is probably due to the fact that the sludge utilized in the experiment whose results are calculated in Table 15 was not adequately acclimated to NTA. This problem has been documented by the work of Pfeil and Lee (1968), who were able to show that NTA will only be degraded by activated sludge which is acclimated to it for at least five days, alternatively, if an acclimated sludge is deprived of NTA for as long as three days, then a period of reacclimation is required. The concentrations of NTA that were finally used

	Reactor	mg Test Compound Removed	mg Solids Added	"Activity" ($\times 10^{-3}$)
S-1 3/17/72	A	16.03	3000	5.0
	B	18.21	3920	5.0
	C	14.15	3000	5.0
	D	15.91	1200	13.0
S-2 3/22/72	A	19.07	3600	5.0
	B	18.00	2800	6.0
	C	11.95	1800	7.0
	D	6.90	308	22.0

TABLE 12. Linear Alkylate Sulfonate Runs. 5.0 mg/l Two Sonication Levels

$$\text{Activity} = \frac{\text{mg test compound removed}}{\text{mg solids added}}$$

	Reactor	mg Test Compound Removed	mg Solids Added	"Activity" ($\times 10^{-3}$)
S-1 3/15/72	A	36.67	4000	9.0
	B	33.90	4000	8.0
	C	28.40	3600	8.0
	D	25.50	1000	26.0
S-2 3/20/72	A	26.42	3000	9.0
	B	23.21	3200	7.0
	C	23.48	1600	15.0
	D	26.78	300	90.0

TABLE 13. Linear Alkylate Sulfonate Runs. 10.0 mg/l Two Sonication Levels

$$\text{Activity} = \frac{\text{mg test compound removed}}{\text{mg solids added}}$$

	Reactor	mg Test Compound Removed	mg Solids Added	"Activity" ($x10^{-2}$)
S-1 5/23/72	A	218.8	3000	7.3
	B	184.5	3000	6.2
	C	127.7	3000	4.3
	D	176.2	260	68.0
S-2 5/24/72	A	249	2200	11.0
	B	249	2200	11.0
	C	142	920	15.0
	D	200	128	156.0

TABLE 14. Nitrilotriacetic Acid Runs. 100 mg/l Two Sonication Level

$$\text{Activity} = \frac{\text{mg test compound removed}}{\text{mg solids added}}$$

	Reactor	mg Test Compound Removed	mg Solids Added	"Activity" ($x10^{-2}$)
S-1 4/26/72	A	93.0	3000	3.1
	B	97.0	3000	3.2
	C	32.0	3000	1.1
	D	84.0	382	22.0
S-2 4/28/72	A	--	--	--
	B	295	2000	15.0
	C	260	584	44.5
	D	92	270	34.1

TABLE 15. Nitrilotriacetic Acid Runs. 150 mg/l Two Sonication Levels

$$\text{Activity} = \frac{\text{mg test compound removed}}{\text{mg solids added}}$$

in our experiments are probably much greater than would be found in a domestic sewage. These concentrations were used because of the difficulty that we had in analyzing for NTA by the photometric procedure of Thompson and Duthie (1968). We finally had to use a titration procedure which gave more consistent results at the higher concentrations. The high "activity" shown in Reactor D in Tables 14 and 15 again indicates the presence of a soluble enzyme.

Ethylene glycol

Although there exist chemical procedures for the determination of glycols, we thought that more specific information might be obtained with greater facility by using an isotopic tracer technique. Ethylene glycol labeled in the 1 and 2 carbon with carbon-14 was used (see Experimental Design and Materials and Methods). The data in Tables 8 and 9 and the "activity" calculations in Tables 16 and 17 indicate that perhaps our system was not acclimated to ethylene glycol. This is, however, refuted by the disappearance of ethylene glycol from the batch experiments of Reactor E (Tables 8 and 9). Our conclusions, based on the known oxidative pathway of ethylene glycol to glycolic acid are that ethylene glycol is actually degraded in our system. However, because of the nature of

Reactor	mg Test Compound Removed	mg Solids Added	"Activity" ($x10^{-3}$)
A	7.9	3400	2.0
B	6.5	3120	2.0
C	1.7	2600	1.0
D	1.0	160	6.0

TABLE 16. Ethylene-1,2-^{14}C-glycol, 1/25/72, 10 mg/l, Initial and Input Concentration

$$\text{Activity} = \frac{\text{mg test compound removed}}{\text{mg solids added}}$$

Reactor	mg Test Compound Removed	mg Solids Added	"Activity" ($x10^{-3}$)
A	1.82	3000	1.0
B	6.50	3000	2.0
C	10.41	1880	6.0
D	0.00	560	0.0
E	5.50	1260	4.0

TABLE 17. Ethylene-1,2-^{14}C-glycol, 1/26/72, 10 mg/l Initial and Input Concentration

$$\text{Activity} = \frac{\text{mg test compound removed}}{\text{mg solids added}}$$

the end products and intermediates (see below) (Reference Doelle, 1969)

CH_2-OH		CHO		COOH
	→		→	
CH_2-OH		CH_2OH		CH_2OH
Ethylene glycol		glycolic aldehyde		glycolic acid

that analysis using either a single or double isotope label cannot detect the aldehyde or acid formed because no break occurs in the primary carbon chain, consequently, there is no loss of isotope even though the test compound may be extensively degraded. Only in the batch system (Figure 23) where the glycolic acid formed is in contact with further oxidation systems, which break the C-C bond and release CO_2, is this degradation obvious. Supportive evidence for this is to be found in the results of reactor C in the extended retention time experiment (Table 17). Further experiments with ethylene glycol were not attempted due to time considerations, although a chemical analytical procedure based on reaction of adjacent hydroxyl groups might show substantially different results.

Ethylene dinitrilotetraacetic acid (EDTA)

Experimental results from EDTA were disappointing. We were never able to acclimate sludge to EDTA. Figure 20 substantiates this position and Figure 19 shows analysis of supernate from sludge being acclimated to EDTA. It appears from Figure 20 that the EDTA in the supernate simply accumulates - thus no experiments could be carried out with EDTA.

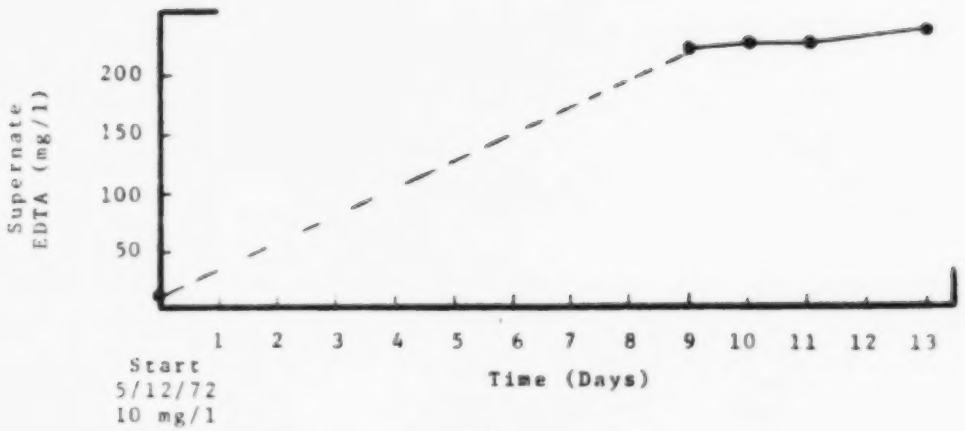

FIGURE 19. Residual EDTA in Sludge Supernate as a Function of Time - To Show Absence of Acclimation. Feed rate - start at 10 mg/l increased by increments to 100 mg/l.

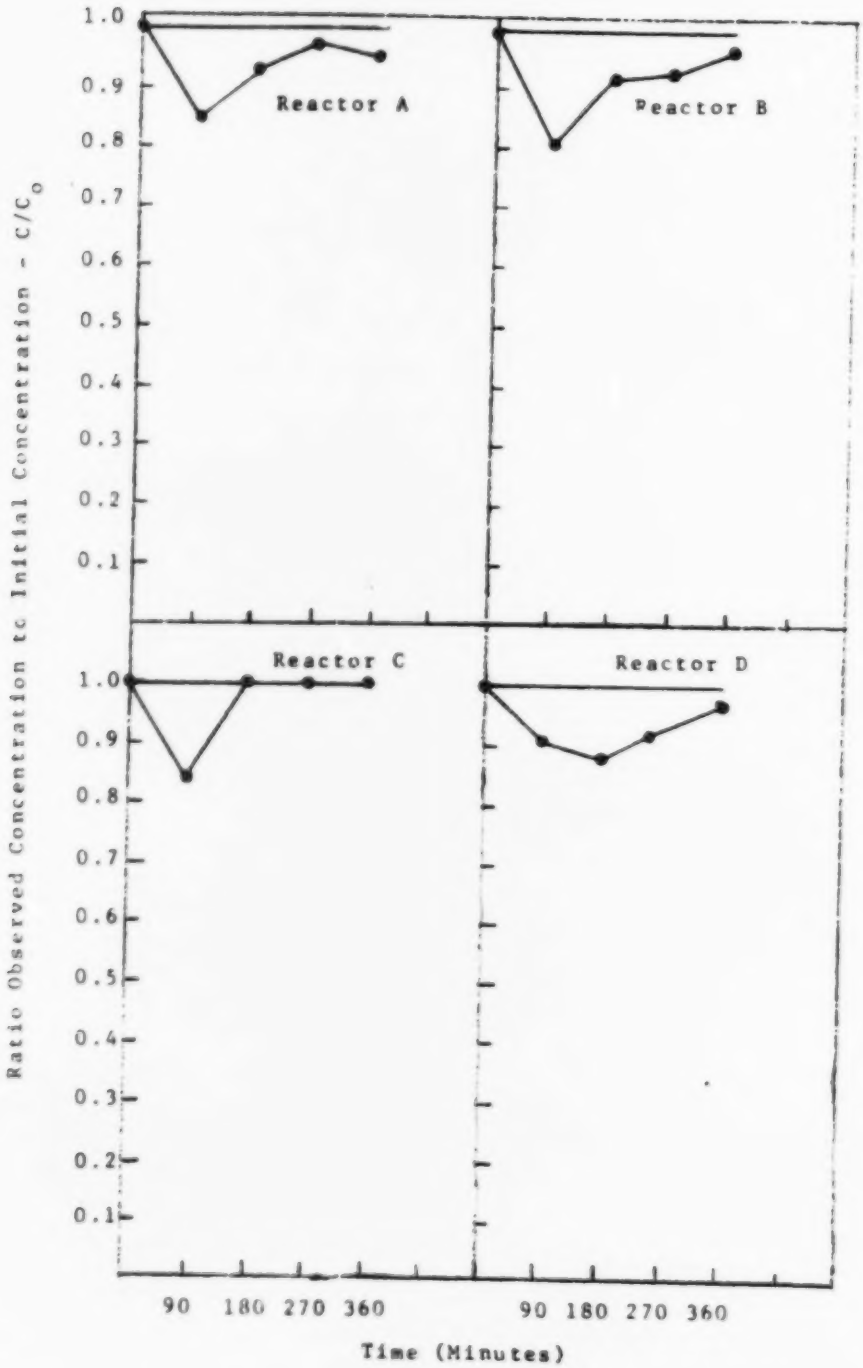

FIGURE 20. EDTA Run, 150 mg/1, S-1, 5/2/72
EDTA Concentration —— Theoretical • Experimental

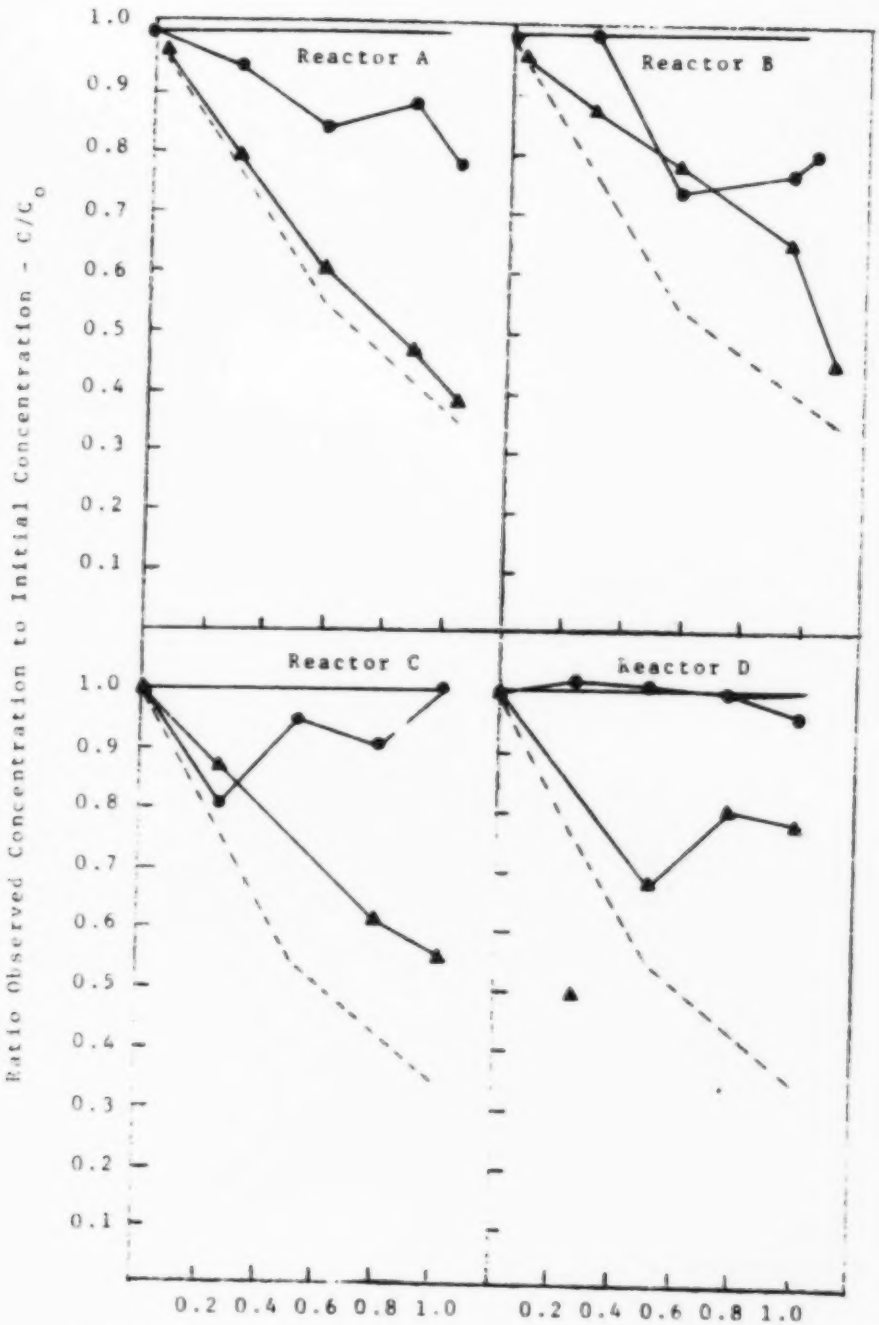

FIGURE 21. Ethylene-1,2-^{14}C-glycol, 10 mg/l, S-1

Ethylene Glycol —— Theoretical ● Experimental
Total Suspended Solids --- Theoretical ▲ Experimental

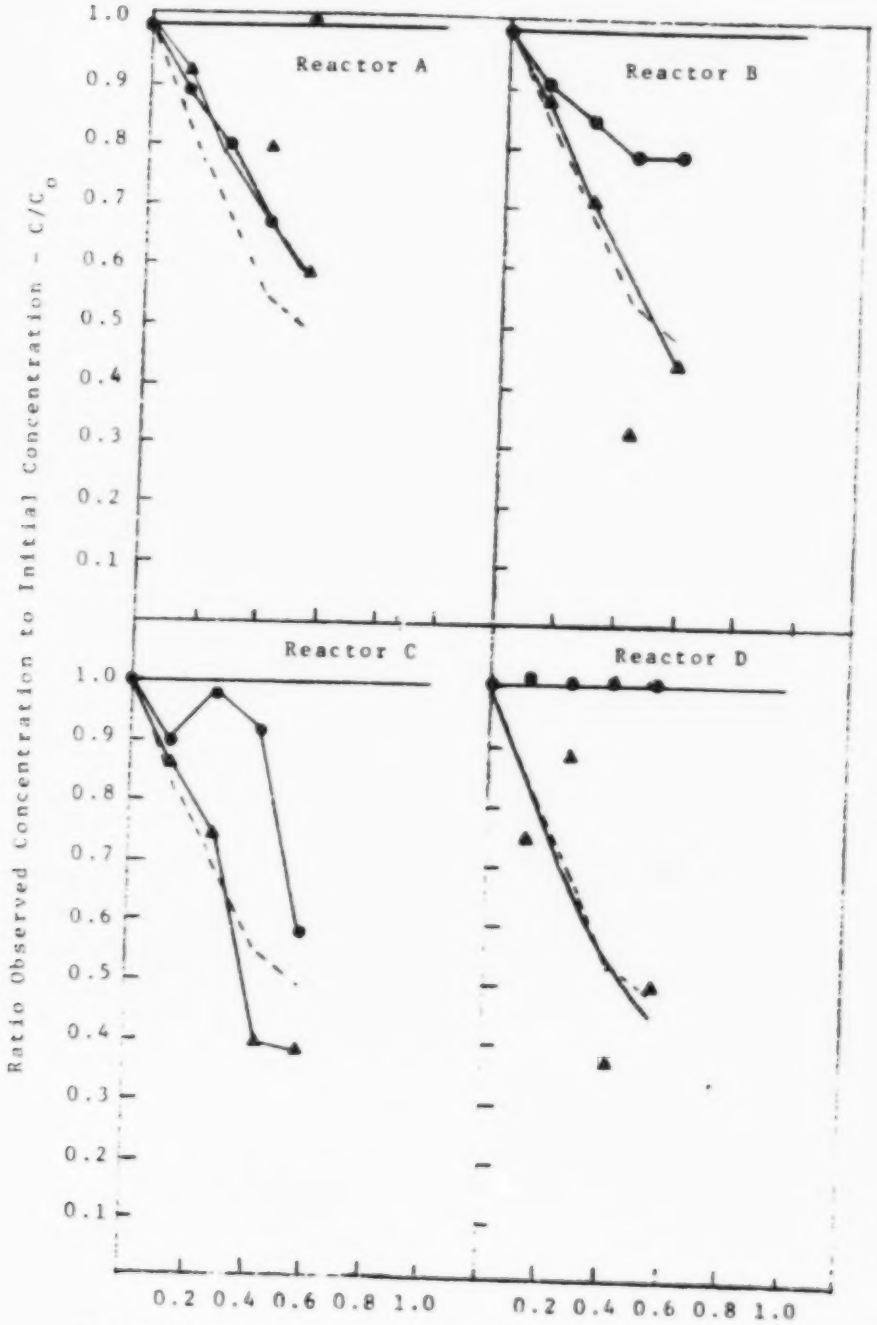

FIGURE 22. Ethylene-1,2-^{14}C-glycol, 10 mg/l, S-1 Extended Retention Time.

Ethylene Glycol —— Theoretical • Experimental
Solids Washout --- Theoretical ▲ Experimental

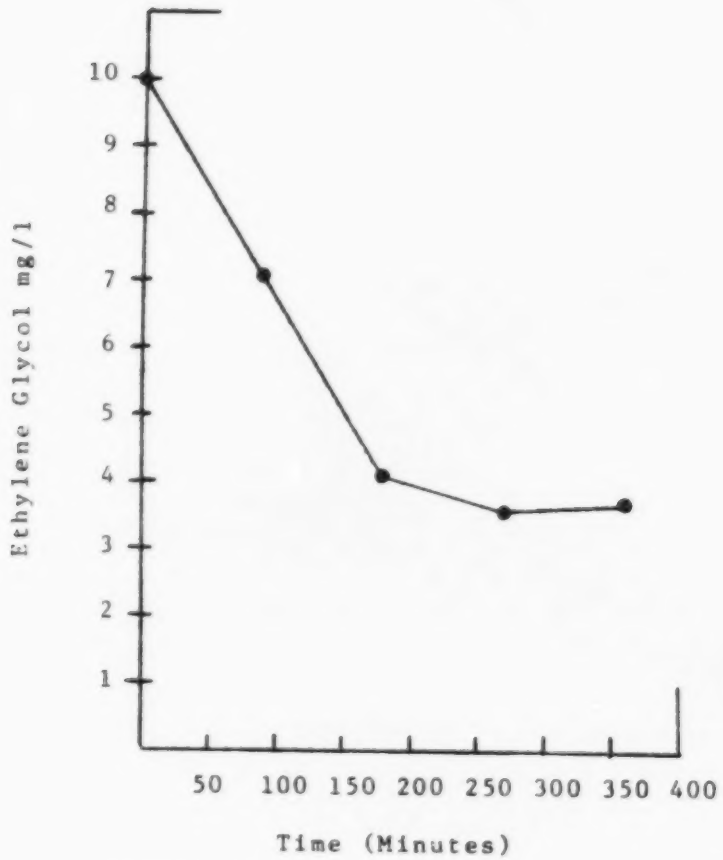

FIGURE 23. Ethylene Glycol Removal ● Batch Run. Start 10 mg/l. Blended Sludge.

Removal Rates

Removal rates were calculated for the linear portions of the degradation curves for DCP, LAS and NTA, these experiments being representative of the best data collected. As can be seen from Table 18, the removal rates are fairly consistent within each experiment.. Reactors C and D are consistent in that one would expect that if inhibition is occurring due to adsorption onto particulate material in C then D would always be higher except in those cases where the enzymes are membrane bound in which instance Reactor D removal rates would be lower. The amount of disruption will also make a difference in C and D for if disruption is only slight, then D should always be lower in rate of removal than C because the enzymes would not have been released and would have been removed with the cells by centrifugation.

TOC Removal

Total Organic Carbon removal appears to follow much the same pattern as the test compounds - unfortunately, no initial measurements of TOC were made, therefore, little basis for comparison exists. It is evident, however, that the initial values were at least of the magnitude of the 90 minute samples (see Table 3). Therefore, one can show that the removal of TOC in reactors C and D in both S-1 and S-2 conditions is at least 30-35% of what appears at the 90 minute sampling period.

Compound	Initial and Input Concentration (mg/l)	Sonication Level	Reactor A	Reactor B	Reactor C	Reactor D
DCP	2.5	S-1	2.76 (64)	2.71 (64)	2.71 (64)	1.89 (44)
DCP	2.5	S-2	3.49 (84)	3.53 (84)	2.50 (60)	3.13 (74)
DCP	5.0	S-1	4.31 (52)	4.57 (52)	4.05 (48)	3.68 (44)
DCP	5.0	S-2	5.79 (70)	5.31 (64)	4.97 (60)	4.13 (50)
LAS	5.0	S-1	3.13 (42)	4.15 (54)	3.63 (50)	3.23 (46)
LAS	5.0	S-2	6.63 (80)	6.66 (80)	5.35 (64)	2.63 (32)
LAS	10.0	S-1	12.78 (77)	13.38 (79)	4.97 (29)	12.21 (73)
LAS	10.0	S-2	9.61 (55)	9.33 (56)	9.18 (56)	4.80 (29)
NTA	100.0	S-1	70.4 (40)	59.76 (36)	26.24 (16)	27.09 (16)
NTA	100.0	S-2	57.17 (35)	69.16 (42)	34.36 (21)	59.27 (35)

TABLE 18. Removal rates in mg test compound per hour for best 3 sets of data. Dichlorophenol (DCP), Linear Alkylate Sulfonates (LAS), Nitrilotriacetic Acid (NTA). The numbers in parentheses are the percentage of total test compound input in 90 min. which are removed in 90 min.

It must, therefore, be greater than 50% of the original value - more likely 75% removal of the original TOC present. However, the final concentration of TOC at the end of the six hour experiment is greater in all cases for reactors C and D than for reactors A and B.

Settling Volume

Table 3 also shows the amount of solids which settle out in 30 minutes, for two experiments at sonication levels of 20 minutes (S-1) and 1 hour (S-2). It is apparent from this data that very little in the way of settleable solids remains in the effluent of reactors C and D for either sonication level. The reduction in settleable solids is approximately 99% in the case of both S-1 and S-2, indicating perhaps a too effective sonication procedure.

SECTION VI

DISCUSSION

Microbiological Processes

Industrial microbiological processes, continuous or batch may be classified as follows according to purpose: (Herbert, D.,1961)

1. Production of microorganisms
2. Effecting desired chemical transformations.

The latter purpose may also be subdivided into:

a. production of a desired end-product

b. decomposition of a given starting material.

Subdivision "a" includes such areas as industrial solvent production, antibiotic production, steroid transformations, etc. Sewage disposal and destruction of industrial wastes are included in subdivision "b".

Biochemically these subdivisions are not important, as all chemical transformation processes may be sharply delineated from processes, the purpose of which is to produce microbial cells. The differentiating feature is that chemical transformations are not of necessity accompanied by growth.

Many chemical transformations can be accomplished by cells that are not growing or even by cells that are no longer alive; these transformations are brought about by the enzymes that the cells contain. Furthermore, the most controllable way of effecting many transformations, and the way which gives the highest yields, is to use resting cell suspensions, or enzymes prepared from them. In spite of this, microbial transformations are most commonly accomplished by adding the starting material to a growing culture. Efficiency might be increased,

The effectiveness of disrupting the bacterial cells prior to their application to the waste water plus test compound may be seen by observing the amount of test compound removed in Reactor C. This is the reactor which presumably contains the released enzymes along with the cellular debris. It is obvious that the absolute amount of substrate removed is never less than 50% of the amount removed in Reactors A and B, and on a per milligram of solids basis is not significantly different than the amount removed per milligram in the first two reactors.

We have attributed part of the difference observed in substrate removal between Reactors C and D to adsorption onto particulate matter. At this point, it might be well to detail the problems associated with controlling the performance of enzymes outside of their normal milieu. Enzymes are found in nature in complex mixtures, usually in cells containing a hundred or more different enzymes. Within the cell, highly organized conditions obtain which keep the enzyme molecules from interfering with each other, and in their proper spatial configurations relative to the reaction(s) which each catalyzes. When cells are disrupted the highly organized state no longer exists, consequently interactions occur between enzyme and enzyme, enzyme and cellular debris or enzyme and external environment. Enzymes may interface with other enzymes, either by attacking the substrate giving rise to side reactions (competition), or by transforming the

product into some other substance thus interrupting a sequence of normal metabolic reactions, or perhaps by attacking the coenzyme or even the enzyme itself (proteases involved in autolysis, etc.).

Once released to the external environment, enzymes are subject to the limitations of any protein molecule influenced by variations in temperature, pH, substrate concentration, ionic strength, and the presence of protein precipitants, and metal ion activators or inhibitors, etc. In spite of the influence of these external variables, it is evident, from the data presented, that the enzymes released by cell disruption into a waste mixture still retain much of their original activity, for a relatively long period. For example, in the experiments shown, Reactor C and D showed no significant rise in the concentration of test compound, indicating that the enzymatic reactions involved in their removal were still operating at the end of six hours. Even though Reactors C and D contained respectively disrupted cells and the supernate from such disrupted cells - at concentrations equivalent to those in Reactors A and B.

It has been the purpose of this investigation to establish that efficiency of activated sludge treatment might be increased by separating the growth (sludge production) from the transformation (substrate removal) steps, in a conventional activated sludge system.

This concept of separation of growth and substrate removal has been amply demonstrated in industry (see above) but has had limited application to waste water treatment. Some recent investigations, however, demonstrate the potential for this approach. For example, in rapid uptake of orthophosphate by activated sludge previously starved for phosphate (WPCRS 17010 DDQ 11/71). Also, Krishnan and Gaudy (1968), have shown that the rate of substrate removal and the pattern of substrate conversion to carbohydrate were substantially the same for systems in which protein synthesis was inhibited (no growth) as for normal systems (growth).

The work reported here is a preliminary study and should be viewed as such. Nevertheless, it has demonstrated that substrate removal for a specific substrate may be as much as 10 times higher for disrupted cell supernate as for undisrupted cells. The study has also brought out that the application of disrupted cells to conventional treatment systems may not be the straightforward process that we considered at the beginning. The application of disrupted microorganisms to conventional waste water treatment processes will have to overcome the adsorption inhibition that was noted in the experimental phase. Also, mixing of the enzyme supernate with conventional activated sludge is an area which deserves investigation. The addition of disrupted cell supernate to the influent waste water itself, prior to its contact with the activated sludge is another

problem area, as well as the relationship between disrupted cell fragments and inhibition of enzymatic activity.

Despite the many problems involved, further study seems to be justified due to the obvious potential applications of the technique.

SECTION VII

ACKNOWLEDGMENT

We should like to thank Mr. Delbert Jeter, Supervisor of the Bellaire, Texas, Waste Treatment Facility and the City of Bellaire, Texas, for allowing us access to their plant.

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SECTION IX

APPENDIX

Analytical Procedures

Dichlorophenol:

Samples collected in a beaker containing 1 ml (1 + 9) H_3PO_4 and 2.5 ml (10% $CuSO_4 \cdot 7H_2O$) to retard biological activity and prevent formation of H_2S which interferes with the analysis. Samples refrigerated until analyzed.

250 ml aliquots were adjusted to pH 4.0 with 1 + 9 H_3PO_4 using a Coleman Metrion III pH meter.

200 ml of this aliquot was distilled - then 50 ml distilled water was added to the distillation flask and another 50 ml distilled over.

To 100 ml of distillate in a 250 ml flask were added 2.0 ml of ammonium chloride solution (5.0%). pH was adjusted to 10.0 with concentrated NH_4OH. Added 2.0 ml of Aminoantipyrine solution (2%) and 2.0 ml of potassium ferricyanide solution (8%). Allow to stand 15 minutes and read on spectrophotometer at 510 nanometers, in a 2.5 cm path length cuvette. Standards: 100 ml phenol standards containing 0.1, 0.2, 0.3, 0.4 and 0.5 mg phenol were run with each analysis - along with a 100 ml distilled water blank.

Linear Alkylate Sulfonates:

Samples collected in presence of Sodium Azide to reduce biological degradation. Samples refrigerated until analyzed.

20 ml aliquots taken and diluted to 100 ml. Solution made alkaline by addition of 1N NaOH to phenol pthalein end point, pink color then discharged by dropwise addition of $1NH_2SO_4$. 25 ml methylene blue reagent added (Standard Methods, p. 340) and 50 ml chloroform. Mixture shaken vigorously for 30 seconds and phases allowed to separate (considerable emulsion formed in the samples containing disrupted cells and supernate). 40-50 ml of the chloroform phase was pipetted using a volumetric pipette and washed with wash solution (Standard Methods, p. 340). After allowing the phases to separate again, 20 ml of the chloroform phase was removed, diluted to 50 ml in a volumetric flask and read against a distilled water blank at 652 nanometers in a 2.5 cm path length cuvette.

Nitrilotriacetic Acid:

Stock solution - Cupric Acetate Monohydrate $(CH_3COO)_2Cu \cdot H_2O$ 3.142 grams/1.

Working solution - 504 ml stock diluted to 1 liter - 1 ml = 2 mg NTA.

0.01M Sodium Acetate solution.

Procedure:

Sample filtered through Whatman #1 or fiber glass plug to remove solids.

50 ml filtered sample + 50 ml 0.01M Sodium Acetate solution. 2-5 drops 0.1% Murexide (in distilled water). Titrate with working solution to yellow endpoint.

Ethylene dinitrilotetraacetic Acid: (Blijenberg & Leijnse, 1969)

Working solution same as for NTA.

Acetate buffer - 600 ml 0.1N Sodium Acetate plus 400 ml 0.1M Acetic acid, check on pH meter, should be pH 4.8, adjust if necessary.

Procedure:

50 ml sample filtered as for NTA.

50 ml acetate buffer pH 4.8.

5-10 drops PAN indicator (pyridylazonapthol) 0.1% solution. Titrate with cupric acetic solution to permanent purple end-point. 1 ml copper acetate = approximately 2.2 mg EDTA.

Ethylene-1,2-^{14}C-glycol Measurements

100µc in 0.2 ml methanol diluted to 10 ml with deionized water = 10µc/ml.

2.81µc added to each reactor.

0.2 ml samples taken at 90 minute intervals added directly to 10 ml of scintillation fluid. After all "samples" had been collected, they were assayed for radioactivity on a Packard Tri-Carb Model 3003 scintillation counter; with our samples 90% efficiency for ^{14}C was achieved.

Scintillation Fluid

42 ml "Liquifluor" - New England Nuclear Corporation.

600 ml reagent grade toluene.

300 ml "Triton-X-100" - Rohm and Haas.